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Date: October 13, 2000

Docket No.: 0055-0310P

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This is a Request for filing a ☐ continuation ☒ divisional ☐ continuation-in-part application under 37 C.F.R. § 1.53(b) of pending prior Application No. 08/448,489 filed on June 7, 1995, the entire contents of which are hereby incorporated by reference,
by

SEIKI, Motoharu; SATO, Hiroshi; and SHINAGAWA, Akira

for

NOVEL METALLOPROTEINASE AND ENCODING DNA THEREFOR

1. ☒ Enclosed is an application consisting of specification, claims, declaration and drawings/photographs (if applicable).

2. ☒ The filing fee has been calculated as follows:

			LARGE ENTITY	SMALL ENTITY
	BASIC FEE		$710.00	$355.00
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	10-20 =	0	x 18 = $0.00	x 9 = $0.00
INDEPENDENT CLAIMS	6-3 =	3	x 80 = $240.00	x 40 = $0.00
☐ MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $270.00	+ $135.00
		TOTAL	$950.00	$0.00

3. ☒ A check in the amount of $950.00 to cover the filing fee and recording fee (if applicable) is enclosed.

4. ☐ Please charge Deposit Account No. 02-2448 in the amount of $0.00. A triplicate copy of this request is enclosed.

5. Amend the specification by inserting before the first line thereof the following:

a. ☐ --This application is a ☐ continuation ☐ divisional ☐ continuation-in-part of co-pending Application No. 08/448,489, filed on June 7, 1995, the entire contents of which are hereby incorporated by reference.--

b. ☒ --This application is a ☐ continuation ☒ divisional ☐ continuation-in-part of co-pending Application No. 08/448,489, filed on June 7, 1995, which is a continuation-in-part application of PCT International Application No. PCT/JP94/02009 filed on November 30, 1994. The entire contents of each of the above-identified applications are hereby incorporated by reference.--

6. ☒ Enclosed is/are fifteen (15) sheet(s) of formal drawings and/or photographs.

7. ☐ A statement claiming small entity status was filed in prior Application No. 08/448,489 on _____. See the attached copy of the statement claiming small entity status.

8. ☒ The prior application is assigned to Fuji Yakuhin Kogyo Kabushiki Kaisha.

9. ☒ A Preliminary Amendment is enclosed.

10a. ☐ Priority of Application No(s). _____ filed in _____ on _____ is/are claimed under 35 U.S.C. § 119. See attached copy of the Letter claiming priority filed in the prior application on _____.

10b. ☒ Priority of International Appln. PCT/JP94/2009 filed on November 30, 1994 under the Patent Cooperation Treaty and Japanese Application No. 5-341061 and 7-109884 filed in JAPAN on November 30, 1993 and March 31, 1995, respectively under 35 U.S.C. § 119 are hereby reclaimed.

11. ☒ An Information Disclosure Statement and PTO-1449 form(s) are attached hereto for the Examiner's consideration.

12. ☒ Address all future communications to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP
P.O. Box 747
Falls Church, VA 22040-0747
Telephone: (703) 205-8000
or
Customer No. 2292

13. ☐ An extension of time for _____( ) month(s) until has been submitted in parent Application No. 08/448,489 in order to establish co-pendency with the present application.

14. ☒ Also enclosed herewith is the following:

Receipt of Deposit of Microorganism

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By Maryanne Armstrong (Reg No 40,069)
Marc S. Weiner, #32,181

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MSW/MAA/csp
0055-0310P

Attachments

(Rev. 09/29/2000)

PATENT
55-310P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: M. SEIKI et al.

Appl. No.: Rule 60 Divisional of 08/448,489 Group: Unassigned

Filed: October 13, 2000 Examiner: Unassigned

For: NOVEL METALLOPROTEINASE AND ENCODING DNA THEREFOR

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

October 13, 2000

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 1

Line 17, after "monoclonal" insert --and polyclonal--

Page 2

Line 12, after "monoclonal" insert --and polyclonal--

Page 3

Line 17, change "Sequence" to --SEQ--

Line 18, change "Sheet sequence number 1" to --ID NO: 1--

Line 22, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Page 4

Line 1, change "Sequence Sheet sequence number" to --SEQ ID NO:--

Line 3, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 17, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 19, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 20, change "Sequence Sheet" to --SEQ ID--

Line 21, change "sequence number 1" to --NO: 1--

Line 23, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 24, change "Sequence Sheet sequence" to --SEQ ID--

Line 25, change "number 1" to --NO: 1--

Page 5

Line 2, change "Sequence Sheet" to --SEQ ID --

Line 3, change "sequence number 2" to --NO: 2--

Line 4, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 6, change "Sequence" to --SEQ--

Line 7, change "Sheet sequence number 1" to --SEQ ID NO: 1--

Line 9, change "(Sequence Sheet" to --(SEQ ID--

Line 10, change "sequence numbers 3 and 4)" to --NOS: 3 and 4)--

Line 14, change "Sequence Sheet sequence numbers 5 and 6" to --SEQ ID NOS: 5 and 6--

Line 21, change "Sequence Sheet" to --SEQ ID--

Line 22, change "sequence number 2" to --NO: 2--

Line 23, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 27, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Page 6

Line 5, change "Sequence Sheet" to --SEQ ID--

Line 6, change "sequence number 1" to --NO: 1--

Line 8, change "Sequence Sheet sequence number 7" to --SEQ ID NO: 7--

Line 20, change "Sequence Sheet sequence number 7" to --SEQ ID NO: 7--

Page 7

Line 14, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 16, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 18, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Page 8

Line 3, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 7, change "Sequence" to --SEQ--

Line 8, change "Sheet sequence number 2" to --ID NO: 2--

Line 13, after "monoclonal" insert --and polyclonal--

Line 16, after "monoclonal" insert --and polyclonal--

Page 11

Line 2, change "Sequence Sheet sequence" to --SEQ ID--

Line 3, change "numbers 3" to --NOS: 3--

Line 10, change "Sequence Sheet sequence numbers " to --SEQ ID NOS:--

Page 15

Line 10, after "XLI-Blue" please insert --(Deposited in the National Institute of Bioscience and Human-Technology as Deposit No. P-15032).--

Line 23, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Line 24, change "Sequence Sheet sequence number 2" to --SEQ ID NO: 2--

Page 16

Line 3, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 4, change "Sequence" to --SEQ--

Line 5, change "Sheet sequence number 2" to --ID NO: 2--

Line 12, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 13, change "Sequence Sheet sequence number 7" to --SEQ ID NO: 7--

Line 17, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 19, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 23, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Page 18

Line 16, change "Sequence" to --SEQ--

Line 17, change "Sheet sequence number 1" to --ID NO: 1--

Line 17, change "Sequence Sheet" to -- SEQ ID--

Line 18, change "sequence numbers" to --NOS:--

Line 18, change "Sequence Sheet" to --SEQ ID--

Line 19, change "sequence number 1" to --NO: 1--

Page 31

Line 1, change "Sequence Sheet sequence number 1" to --SEQ ID NO: 1--

Line 4, change "Sequence Sheet sequence" to --SEQ ID--

Line 5, change "number 1" to --NO: 1--

Page 36

Line 7, after "MT-MMP" insert --(SEQ ID NO: 1)--

Line 8, after "1" (first occurrence) insert --(SEQ ID NO: 12)--

Line 8, after "-2" insert --(SEQ ID NO: 17)--

Line 8, after "-3" insert --(SEQ ID NO: 15)--

Line 8, after "-7" insert --(SEQ ID NO: 18)--

Line 8, after "-8" insert --(SEQ ID NO: 13)--

Line 8, after "-9" insert --(SEQ ID NO: 16)--

Line 8, after "-10" insert --(SEQ ID NO: 14)--

Line 8, after "-11" insert --(SEQ ID NO: 11)--

Please replace pages 38 through 50 of the specification with the substitute sequence listing enclosed herewith. Please renumber the remaining pages of the specification, beginning with the claims.

Please insert the substitute sequence listing attached hereto immediately after the Abstract of the Disclosure.

IN THE CLAIMS:

Please cancel claim(s) 1-12 without prejudice or disclaimer to the subject matter contained therein.

Please add the following new claims:

--1. A DNA having the nucleotide sequence shown in SEQ ID NO: 2 which corresponds to the amino acid sequence of a membrane-type matrix-metalloproteinase characterized by a continuous sequence of hydrophobic amino acids peculiar to membrane-binding proteins from amino acid number 533 to 562 in the C terminus domain shown in SEQ ID NO: 1, having the amino acid sequence from amino acid number 160 to 173, 320 to 333 and from 498 to 512 shown in SEQ ID NO: 1 or having the amino acid sequence from amino acid number 1 to 173, 320 to 333, 498 to 512 and 563 to 582 shown in SEQ ID NO: 1.

2. A plasmid containing a DNA according to claim 1 having the nucleotide sequence shown SEQ ID NO: 2.

3. A host cell harboring a plasmid according to claim 2 containing a DNA having the nucleotide sequence shown in SEQ ID NO: 2.

4. Antibodies which specifically recognize a membrane-type matrix-metalloproteinase characterized by a continuous sequence of hydrophobic amino acids peculiar to membrane-binding proteins from amino acid number 533 to 562 in the C terminus domain shown in SEQ ID NO: 1, having the amino acid sequence from amino acid number 160

to 173, 320 to 333 and from 498 to 512 shown in SEQ ID NO: 1 or having the amino acid sequence from amino acid number 1 to 173, 320 to 333, 498 to 512 and 563 to 582 shown in SEQ ID NO: 1.

5. Antibodies according to claim 4, wherein said antibodies are monoclonal antibodies.

6. A DNA having the nucleotide sequence shown in SEQ ID NO: 2 which encodes a protein having the amino acid sequence shown in SEQ ID NO: 1.

7. A plasmid containing a DNA having the nucleotide sequence shown in SEQ ID NO: 2, expressing the protein shown in SEQ ID NO: 1.

8. A host cell harboring a plasmid containing a DNA having the nucleotide sequence shown in SEQ ID NO: 2, and expressing the protein shown in SEQ ID NO: 1.

9. Antibodies which specifically recognize a protein having the amino acid sequence shown in SEQ ID NO: 1.

10. Antibodies according to claim 9, wherein said antibodies are monoclonal antibodies.--

REMARKS

The specification has been amended to recite "and polyclonal." Support for polyclonal antibodies may be found in working Examiner 3(c) in that the antibodies produced in the mouse and the isolated antibody producing spleenocytes are polyclonal in nature. In addition, the hybridoma cultures of Working Example 3(e) produce polyclonal antibodies.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Applicants' further respectfully request that the disk containing the amended sequence listing which was filed in parent application No. 08/448,489 on February 26, 1999 as file name "55-290p.app" be transferred to this new application in compliance with 37 C.F.R.§ 1.821-825. The disk copy of the sequence listing is identical to the paper copy except for word processing formatting.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, Ph.D. (Reg. 40,069) at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By MaryAnne Armstrong (Reg No 40,069)
for Marc S. Weiner, #32,181

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MSW/MAA/csp
55-310P

(Rev. 04/19/2000)

DESCRIPTION

Title of the Invention

NOVEL METALLOPROTEINASE AND ENCODING DNA THEREFOR

TECHNICAL FIELD

The present invention relates to a novel metalloproteinase useful in applications such as diagnosis of the presence of tumour cells, diagnosis of the degree of tumour malignancy, or other medical or physiological fields.

More specifically, the present invention relates to one type of metalloproteinase expressed specifically in human tumour cells and a DNA sequence encoding therefor; a plasmid having a nucleotide sequence which contains said DNA sequence; a host cell harbouring said plasmid; a method for manufacturing said protein using said host cell; a probe which hybridizes with the aforesaid DNA sequence; a method for detecting DNA or RNA containing the aforesaid sequence using said probe; and monoclonal antibodies which bind specifically to the aforesaid protein.

BACKGROUND

A group of enzymes with different substrate specificity and referred to in general as matrix metalloproteinases (hereinafter referred to as "MMPs") contributes to degradation of the extracellular matrix comprising such

complex components as collagen, proteoglycan, elastin, fibronectin, and laminin.

Previously reported MMPs include interstitial collagenase (MMP-1), 72 kDa gelatinase (also known as type IV collagenase or gelatinase A; MMP-2), 92 kDa gelatinase (also known as type IV collagenase or gelatinase B; MMP-9), stromelysin-1 (MMP-3), matrilysin (MMP-7), neutrophil collagenase (MMP-8), stromelysin-2 (MMP-10) and stromelysin-3 (MMP-11).

These MMPs are a family of enzymes whose primary structure has been reported previously. With the exception of MMP-7, the primary structure among the family of reported MMPs comprises essentially an N-terminal propeptide domain, a Zn^+ binding catalytic domain and a C-terminal hemopexin-like domain. In MMP-7 there is no hemopexin-like domain. MMP-2 and MMP-9 contain an additional gelatin-binding domain. In addition, a proline-rich domain highly homologous to a type V collagen $\alpha 2$ chain is inserted in MMP-9 between the Zn^+ binding catalytic domain and the C-terminal hemopexin-like domain.

In highly metastatic tumour cells, there are reports of conspicuous expression of type IV collagenase (MMP-2, MMP-9) which mainly degrade type IV collagen (Cancer Res., 46:1-7, 1986; Biochem. Biophys. Res. Commun., 154:832-838, 1988; Cancer, 71:1368-1383, 1993). Likewise, it has been reported MMP-3 act as an activator of proMMP-9 (J. Biol. Chem., 267:3581-3584, 1992).

The degree of matrix metalloproteinase expression serves as an index to diagnosing the degree of cancer malignancy.

DISCLOSURE OF THE INVENTION

The present inventors discovered a novel matrix metalloproteinase (hereinafter referred to as "MT-MMP") and performed a structural analysis thereof.

As described hereafter, the present invention offers a novel metalloproteinase protein, DNA having a nucleotide sequence which encodes said protein, a plasmid having said DNA nucleotide sequence, a host cell harbouring said plasmid and monoclonal antibodies which specifically recognize the aforesaid metalloproteinase protein.

1. A native membrane-type matrix-metalloproteinase characterized by a continuous sequence of hydrophobic amino acids peculiar to membrane-binding proteins from amino acid number 533 to 562 in the C terminus domain shown in Sequence Sheet sequence number 1.

2. A native membrane-type matrix-metalloproteinase according to claim 1, characterized by the amino acid sequence from amino acid number 160 to 173, 320 to 333 and from 498 to 512 shown in Sequence Sheet sequence number 1.

3. A native membrane-type matrix-metalloproteinase according to claim 1, characterized by the amino acid sequence from amino acid number 1 to 173, 320 to 333, 498 to

512 and 563 to 582 shown in Sequence Sheet sequence number 1.

4. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, which corresponds to the amino acid sequence of a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

5. A plasmid containing a DNA having the nucleotide sequence according to claim 4 and expressing a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

6. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence according to claim 4, and expressing a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

7. Monoclonal antibodies which peculiarly recognize a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

8. A protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

9. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2 which encodes a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

10. A plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.

11. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.

12. Monoclonal antibodies which peculiarly recognize a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

The present invention is described in detail hereafter.

Using highly conserved sequences (Sequence Sheet sequence numbers 3 and 4) selected from amino acid sequences of the known matrix metalloproteinase (MMP) family, the present inventors designed and synthesized an oligonucleotide primer having the sequences denoted by Sequence Sheet sequence numbers 5 and 6. A PCR was carried out using said oligonucleotide primer and a human placental cDNA library, the PCR products obtained were sequenced, and a 390 bp DNA fragment having a sequence non-homologous to known MMP was obtained. Using this 390 bp DNA fragment as a probe, the human placenta cDNA library was screened, and a cDNA in the positive phage clone obtained was sequenced. The nucleotide sequence is that denoted by Sequence Sheet sequence number 2. A sequence identical to the nucleotide sequence in Sequence Sheet sequence number 2 did not exist in the Genbank/EMBL DNA database, and DNA having this nucleotide sequence was ascertained to be completely novel.

The nucleotide sequence of the aforesaid cloned cDNA in Sequence Sheet sequence number 2 had a 3' non-coding

sequence and open reading frame that potentially encode 582 amino acid. An initiation codon was located at nucleotide number 112, and a stop codon was present at nucleotide number 1858. It was determined that this open reading frame encoded the 582 amino acid sequence in Sequence Sheet sequence number 1, that a deduced signal sequence continued after the initiation codon, and that a hydrophobic domain (Sequence Sheet sequence number 7) specific to a membrane-binding protein of 20 or more linked hydrophobic amino acids was present from C-terminal amino acid number 533 to 562.

When homology between the amino acid sequence of MT-MMP and that of the known MMP family was analyzed, MT-MMP had high homology to the known MMP family, as shown in Figure 2. The sequences best conserved in MT-MMP were active site sequences, as well as sequences proximal to processing site between precursor and mature substance conserved in the MMP family. The fact that MT-MMP has the structural characteristics of a membrane-binding protein, and the presence in MT-MMP of a sequence of linked hydrophobic amino acids (shown in Sequence Sheet sequence number 7) not found in the rest of the MMP family, strongly suggested that MT-MMP, unlike other MMP family, is a membrane-binding MMP.

When MT-MMP expression in various human tissues was studied by Northern Blot analysis with various tissue-derived Poly(A)RNA, high expression was seen in the placenta, lung and kidney (see Figure 3). Likewise, results from Northern Blot analysis performed with RNA extracted

from normal and tumour areas of human lung squamous cell carcinoma showed that MT-MMP is expressed peculiarly at tumour sites (see Figure 4).

Finally, immunoprecipitation and immunostain experiments using anti-MT-MMP monoclonal antibodies showed that the MT-MMP pertaining to the present invention is expressed on a cell membrane without secretion of a gene product, and MMP-2 activation induced by the expression of MT-MMP was observed in the cells transfected with MT-MMP gene (Nature, 370:61-65, 1994).

Due to the achievements of the above-discussed research by the present inventors, the present invention offers a novel matrix metalloproteinase protein having the amino acid sequence in Sequence Sheet sequence number 1.

In addition, the present invention offers DNA having the nucleotide sequence in Sequence Sheet sequence number 2, which encodes a protein having the amino acid sequence in Sequence Sheet sequence number 1; a plasmid containing and capable of expressing said DNA; and a host cell harbouring said plasmid. All host cells used in general recombinant DNA technology can be used as the aforementioned host cell, including prokaryotes such as E. coli and Baci lus subtilus; eukaryotes such as yeast, COS cells, CHO cells and 3T3 cells; and insect cells such as Sf21. Expression vectors corresponding to used host cells can be used as the aforementioned plasmid.

Furthermore, the present invention offers mRNA transcribed from DNA having the nucleotide sequence in Sequence Sheet sequence number 2.

The present invention also offers a probe which hybridizes with the aforementioned DNA or RNA and specifically detects said DNA or RNA, and said probe may be one having any part of the nucleotide sequence in Sequence Sheet sequence number 2, provided said probe is labeled by a generally used radioactive isotope or enzyme or the like, hybridizes specifically with said DNA or RNA in general blotting analysis and *in situ* hybridization, and accomplishes detection.

Furthermore, the present invention offers monoclonal antibodies which bind peculiarly with the MT-MMP pertaining to the present invention.

The monoclonal antibodies pertaining to the present invention can be prepared by a well-known method such as the method of Milstein et al. (Nature, 256:495-497, 1975) using human MT-MMP as an antigen. In this method, the antigen may be native human MT-MMP, recombinant human MT-MMP, or a synthetic peptide having a partial amino acid sequence of either.

By means of the present invention, DNA having a nucleotide sequence which encodes a protein with the amino acid sequence of the novel MT-MMP pertaining to the present invention can be cloned, and such DNA and a protein encoded by such DNA can be prepared by a genetic engineering

technique. Through the use of a cDNA clone of such a novel MT-MMP, techniques generally used in genetic engineering can be used to clone the aforementioned nucleotide sequence into another vector or host. Based on the aforementioned cDNA nucleotide sequence, DNA appropriately suited to a probe may be designed and prepared. In addition, based on the nucleotide sequence of the MT-MMP pertaining to the present invention, techniques generally used in genetic engineering can be used to prepare a corresponding protein wherein appropriate mutation have been introduced into the MT-MMP amino acid sequence by substitution, deletion, insertion, displacement or addition of one or more amino acids. All such aforementioned derivatives may also be included in the present invention, provided that common metalloproteinase characteristics are conserved; namely, sequences proximal to processing site between precursor and mature substance, active site sequences and domain structure, and provided that the MT-MMP characteristic of a hydrophobic domain of linked hydrophobic amino acids present near the C terminus is conserved.

Use of the above-discussed various implementations of the present invention offers various technical means applicable to applications pertaining to diagnostic agents or diagnostic methods used for diagnosis of the presence of tumour cells or for diagnosis of the degree of tumour malignancy, as well as applications in other medical or physiological fields.

The present invention is described in detail hereafter by means of Working Examples, but the present invention is not limited by these Working Examples.

WORKING EXAMPLES

Working Example 1 Isolation of novel metalloproteinase (MT-MMP) cDNA

(a) Construction of cDNA Library

Total RNA was extracted from human placenta tissue by a guanidine-cesium chloride method (Biochemistry, 18:5294-5299, 1979) and poly(A)$^+$RNA was purified using an oligo(dT)-cellulose column. Using a purified poly(A)$^+$RNA as a template and an oligo(dT) primer, cDNA was synthesized according to the Gubler-Hoffman method (Gene, 25:263-269, 1983). The ends of the cDNA were converted to blunt end with T_4 DNA polymerase, and EcoR I sites present in the cDNA were methylated by EcoR I methylase. Using T_4 DNA ligase, an EcoR I linker [d(pG-G-A-A-T-T-C-C)] and the cDNA were ligated, and cDNA possessing EcoR I sites at both ends was generated by EcoR I digestion. Using T_4 DNA ligase, this cDNA was cloned into EcoR I site of λgt11. *In vitro* packaging of this cDNA was carried out, for example, using an *in vitro* packaging kit (Amersham), and a cDNA library was thus constructed. A commercial cDNA library such as a human placenta cDNA library (Clontech) can be used as a cDNA library.

(b) Preparation of synthetic oligonucleotide primer

The sequences denoted by Sequence Sheet sequence numbers 3 (P-1) and 4 (P-2) were selected from among amino acid sequences of the known MMP family as highly conserved amino acid sequences in the MMP family, and oligodeoxynucleotide primers corresponding respectively to oligopeptide P-1 and oligopeptide P-2 were designed. Specifically, when amino acids coded by two or more codons were present in an oligopeptide, the sequences were designed as a mixture as shown in Sequence Sheet sequence numbers 5 (primer 1) and 6 (primer 2). Primer 1 and primer 2 were synthesized by a β-cyanoethyl phosphoamidite method using a DNA synthesizer (Applied Biosystems Model 392). Using a NICK column (Pharmacia) equilibrated with 10mM sodium phosphate buffer, pH 6.8 the obtained primer 1 and primer 2 were purified.

(c) Gene amplification by PCR

Using a human placenta-derived cDNA as a template and primers 1 and 2 noted in the above section (b), a PCR (PCR Technology, Stockton Press, pp. 63-67, 1989) was run.

As a result, a 390 bp PCR product was yielded. The obtained PCR product was cloned in an appropriate plasmid, e.g., pUC 119 or pBluescript, and the nucleotide sequence of the PCR product was determined using a fluorescence DNA sequencer (Applied Biosystems, Model 373A) and a Taq dye-primer cycle sequencing kit (Applied Biosystems). Among

various PCR products whose nucleotide sequences were determined, PCR product A having no homology to nucleotide sequences of previously reported MMPs was obtained. PCR product A was used as a probe for screening the human placenta cDNA library noted in the foregoing section (a). ^{32}P labeling of the probe was generated using a random primed DNA labeling kit (Boehringer Mannhaim).

(d) Screening of novel MMP gene from cDNA library and DNA sequencing.

Host E. coli Y1090 was transfected with the human placenta cDNA library constructed in the λgt11 cited in the foregoing section (a) and plaques were formed. Specifically, Y1090 was cultured overnight in an L broth containing 0.02% maltose, and bacteria were harvested and suspended in 10mM $MgSO_4$. This cell suspension and a phage solution were mixed, incubated at 37°C for 15 minutes, and then the phages were adsorbed onto the host bacteria. Soft agar was added thereto, and the material was spread on an L plate (the above-noted operation is hereinafter termed "plating"). The plate was incubated overnight at 42°C and a plaque was formed, after which a nylon filter (e.g., Hibond-N, Amersham) or a nitrocellulose filter (e.g., HATF, Millipore) was placed onto the plate and left in place for approximately 30 seconds. The filter was gently peeled and immersed in an alkaline denaturant (0.1M NaOH, 1.5M NaCl) for 30 seconds, then immersed in a neutralizing solution

(0.5M Tris-HCl buffer, pH 8 containing 1.5M NaCl) for 5 minutes. The filter was then washed with 2x SSPE (0.36M NaCl, 20mM NaH_2PO_4, 2mM EDTA) and dried. The foregoing plaque-to-filter transfer was repeated, and at least two filters were prepared. However, plate contact time for the second and subsequent filters was extended to approximately 2 minutes. Filters were baked 2 hours at 80°C and DNA was thus fixed. The two filters, at a minimum, prepared from one plate were respectively washed 1 hour at 42°C in a wash solution (50mM Tris-HCl buffer, pH 8.0 containing 1M NaCl, 1mM EDTA and 0.1% SDS), placed in a hybridization bag, and prehybridization was carried out by 6 to 8 hours immersion at 42°C in a prehybridization solution [50% formamide, 5x Denhardt's solution (0.2% bovine serum albumin, 0.2% polyvinylpyrolidone), 5x SSPE, 0.1% SDS, 100μg/ml heat-denatured salmon sperm DNA]. Next, the ^{32}P-labeled probe noted in section (c), heat-denatured for 5 minutes at 100°C, was added to the prehybridization solution, and hybridization was carried out overnight at 42°C. After hybridization was complete, the filters were washed at room temperature with an excess of 2x SSC solution containing 0.1% SDS. Next, the filters were placed for 15 minutes at 68°C in 1x SSC solution containing 0.1% SDS. The filters were then dried, layered with X-ray film (Kodak XR), and 1 week autoradiography was then carried out at -70°C. The X-ray films were developed, replica filters in duplicate produced from one plate were piled up each other, and

signals that appeared precisely same place on duplicate filters were marked. Plaques corresponding to marked signals were suspended in SM solution (50mM Tris-HCl buffer, pH 7.5 containing 0.1M NaCl and 10mM $MgSO_4$). These phage suspensions were appropriately diluted and plating was performed, screening similar to that noted above was carried out, and recombinant phages were obtained.

(e) Preparation of recombinant λgt11 DNA

Each cloned phages was plated, incubated for 3 hours at 42°C, and incubated overnight at 37°C. Several drops of chloroform was then added to the SM solution and the material was left at room temperature for 30 minutes. The SM solution together with the upper layer of soft agar was then scraped off, and centrifuged. Polyethylene glycol was added to a 10% final concentration in the supernatant, and the material was mixed and left at 4°C for 1 hour. The material was then centrifuged, the supernatant was discarded, and phage particles were collected. The phage particles were suspended in SM solution and purified by a glycerol gradient ultracentrifugation method (see "Molecular Cloning, a Laboratory Manual", T. Maniastis et al., Cold Spring Harbor: Laboratory Press pp. 2.78, 1989). The phages obtained were suspended in SM solution and treated with DNase I and RNase A. A mixture of 20mM EDTA, 50μg/ml proteinase K, and 0.5% SDS was then added, and the material was incubated at 65°C for 1 hour. The material was then

subjected to phenol extraction and diethylether extraction, and DNA was precipitated by ethanol precipitation. The DNA obtained was washed with 70% ethanol, dried, and dissolved in TE solution (10mM Tris-HCl buffer, pH 8 containing 10mM EDTA).

(f) Sequencing of the insertion fragment

The λgt11 DNA prepared in the above section (e) was digested with EcoR I, an insertion fragment was excised and purified, and cloned into the EcoR I site of a pBluescript (Stratagene) vector. E. coli NM522 XLI-Blue was transformed with this recombinant pBluescript. The F' transformed cells were selected, infected with helper phage VCSM13 (Stratagene), and cultured overnight. The culture was centrifuged and the bacteria were removed, and PEG/NaCl was added to precipitate the phages. The precipitate was suspended in TE solution, and single-stranded DNA was extracted with phenol and recovered by ethanol precipitation. The single-stranded DNA was sequenced using a fluorescence DNA sequencer (Applied Biosystems, Model 373A) and a Taq dye-primer cycle sequencing kit (Applied Biosystems). The total length of the sequence determined was 3403 base pairs, and the sequence thereof is denoted by Sequence Sheet sequence number 2. The nucleotide sequence in Sequence Sheet sequence number 2 was searched using the Genbank/EMBL DNA database, but an identical sequence did not exist.

(g) Analysis of Gene Product

Hydrophilic and hydrophobic values of the amino acid sequence denoted by Sequence Sheet sequence number 1, as predicted from the nucleotide sequence denoted by Sequence Sheet sequence number 2, were calculated by the Kyte-Doolittle method (J. Mol. Biol., 157:105-132, 1982), and the hydrophilic and hydrophobic distribution plot shown in Figure 1 was determined. A hydrophobic domain comprising a sequence of 20 or more linked hydrophobic amino acids peculiar to a membrane binding protein was present from position 533 to position 562 of the C-terminal region of Sequence Sheet sequence number 1, and the sequence thereof is shown in Sequence Sheet sequence number 7. Such a sequence of linked hydrophobic amino acids does not exist in previously known MMPs.

When the homology of the amino acid sequence in Sequence Sheet sequence number 1 was compared to reported MMPs amino acid sequences, the amino acid sequence in Sequence Sheet sequence number 1 showed homology with the MMP family. Specifically, processing site between precursor and active enzyme and active site conserved to an extremely high degree among MMP family were each highly conserved in MT-MMP as well (Sequence Sheet sequence number 1, amino acids numbers 88-97 and 112-222).

Working Example 2 Gene Expression

(a) Expression in Tissues

Using ^{32}P-labeled PCR product A noted in Working Example 1, section (c) as a probe, hybridization was performed with poly(A)$^+$ RNA blotted membrane, human multiple tissue Northern Blots (Clontech), which contains poly(A)$^+$ RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Human multiple tissue Northern Blot filters wetted with 3x SSC (0.45M NaCl, 0.045M trisodium citrate•$2H_2O$, pH 7.0) were prehybridized for 2 to 3 hours in a prehybridization solution (0.75M NaCl, 2.5mM EDTA, 0.5x Denhardt's solution, 50% formamide and 20mM Tris-HCl buffer, pH 7.5 containing 1% SDS) with gentle agitation. Next, a heat-denatured probe was added to the hybridization solution (10% sodium dextran and 50μg/ml denatured salmon sperm DNA-containing prehybridization solution), the prehybridization solution was replaced, and hybridization was performed overnight at 43°C. After hybridization was complete, the filters were washed with 2x SSC containing 0.1% SDS. Next, the filters were placed for 15 minutes at 68°C in 1x SSC containing 0.1% SDS. The filters were then dried, layered with X-ray film (Kodak XR), and 1 week autoradiography was then carried out at -70°C. The size of the MT-MMP gene transcripts was 4.8 kb in each tissue. When the developed X-ray films were traced by a densitometer and signal intensity was measured, among the investigated tissues, MT-MMP genes were found to be highly expressed in the lung, placenta and kidney.

(b) Expression in Tumour Tissues

Normal and tumour tissues were taken from samples of two squamous cell carcinomas human lung, respectively, and total RNA was extracted by a guanidine-cesium chloride method. 10μg of each said RNA was applied to 1% agarose electrophoresis and then transferred onto a nylon membrane. Hybridization was then carried out with the ^{32}P-labeled probe noted in Working Example 1, section (c). Hybridization and autoradiography tracing were performed as described in the foregoing section (a). In each human lung squamous cell carcinoma, significantly higher expression were seen in tumour tissue (see Figure 4 T) than in normal tissue (see Figure 4 N).

Working Example 3 Preparation of Monoclonal Antibodies

(a) Preparation of Polypeptides as Antigen

From the MT-MMP amino acid sequence denoted by Sequence Sheet sequence number 1, sequences denoted by Sequence Sheet sequence numbers 8, 9 and 10 (sequence of Sequence Sheet sequence number 1 amino acid numbers 160-173, 320-333, and 498-512, respectively; hereinafter termed polypeptide A, polypeptide B and polypeptide C, respectively) were selected as specific sequences having low homology to other members of MMP family. These polypeptides were synthesized by Fmoc-BOP method using a peptide synthesizer (MilliGen/Biosearch, Peptide Synthesizer 9600), and cysteine was introduced at

the N-terminus. Each synthesized peptide was purified by high speed liquid chromatography.

(b) Preparation of Each Polypeptides and Keyhole Limpet Hemocyanin Complexes

2 mg of keyhole limpet hemocyanin (KLH) dissolved in 1 ml of 0.1M phosphate buffer, pH 7.5 and 1.85 mg N-(ε-maleimidocaproyloxy)succinimide dissolved in 200 µl dimethylformamide were mixed and incubated at 30°C for 30 minutes. Next, the above-noted mixture was applied to gel filtration by PD-10 (Pharmacia) equilibrated with 0.1M phosphate buffer, pH 7.0. KLH-bound maleimide was collected and concentrated to less than 1.5 ml. Each polypeptide synthesized in the foregoing section (a) was respectively dissolved in 1 ml of 0.1M phosphate buffer, pH 7.0 and mixed with KLH-bound maleimide at a molar ratio representing a factor of 50. This material was then incubated 20 hours at 4°C, and KLH-polypeptide complexes were thus prepared.

(c) Preparation of Antibody-producing Cells

As an initial immunization, eight-week-old Balb/c female mice were given an intraperitoneal administration of 250 µg of a complex of KLH and, respectively, polypeptide A, polypeptide B or polypeptide C prepared in the above section (b), in Freund's complete adjuvant. After 18 days, the respectively immunized mice were boosted intraperitoneally with 200 µg of the respective complexes dissolved in 0.1M

phosphate buffer, pH 7.5. After 32 days, a final immunization of 100 μg of each complex was administered intravenously as the booster immunization. Three days thereafter, splencytes were extirpated and splencyte suspensions were prepared.

(d) Cell Fusion

Fusion with 8-azaguanine-resistant myeloma cell SP2 (SP2/O-Ag14) was performed according to a modifying method of Oi et al (see Selected Methods in Cellular Immunology, Mishell, B.B. and Shiigi, S. M., ed., W.H. Freeman and Company pp. 351-372, 1980). Fusion of myeloma cell SP2 with karyo-splencytes from mice immunized with the polypeptide A-KLH complex is discussed in details, hereafter.

Through the following procedures, karyo-splencytes prepared in the foregoing section (c) (cell viability 100%) were fused in a 5:1 ratio with myeloma cells (cell viability 100%). A polypeptide A-immunized splencyte suspension and myeloma cells were separately washed in RPMI 1640 medium. The material was then suspended in the same medium, and $3x10^8$ cells of karyo-splencytes and $6x10^7$ cells of myeloma cells were mixed for fusion. The cells were then precipitated by centrifugation, and all the supernatant was completely discarded by suction. 2.0 ml of PEG 4000 solution (RPMI 1640 medium containing 50% [w/v] polyethylene glycol 4000) prewarmed at 37°C was added dropwise to the precipitated cells over 1 minute, 1 minute stirring was

performed, and the cells were resuspended and dispersed. Next, 2.0 ml of RPMI 1640 medium prewarmed at 37°C was added in a dropwise fashion over 1 minute. After repeating the same operation once more, 14 ml of RPMI 1640 medium was added dropwise over 2 to 3 minutes under constant stirring, and the cells were dispersed. The dispersion was centrifuged and the supernatant was completely discarded by suction. Next, 30 ml of NS-1 medium (RPMI 1640 medium containing filter-sterilized 15% [w/v] fetal calf serum [JRH Biosciences]) prewarmed at 37°C was rapidly added to the precipitated cells, and the large cell clumps were carefully dispersed by pipetting. The dispersion was then diluted by adding 30 ml of NS-1 medium, and 6.0×10^5 cells/0.1 ml/well was added to a polystyrene 96-microwell plate. The above-noted cell-filled microwells were cultured in 7% carbonic acid gas/93% atmospheric air at 37°C and 100% humidity.

In the case of splencytes derived from mice immunized with the polypeptide B-KLH complex, 6.4×10^8 cells of splencytes and 1.28×10^8 cells of myeloma cells were mixed, and respectively, 4.3 ml, 38.7 ml and 129 ml of the above-used PEG 4000 solution, RPMI 1640 medium and NS-1 medium were used. In the case of splencytes derived from mice immunized with the polypeptide C-KLH complex, 6.8×10^8 cells of splencytes and 1.36×10^8 cells of myeloma cells were mixed, and 4.5 ml, 40.5 ml and 135 ml of respectively PEG 4000 solution, RPMI 1640 medium and NS-1 medium were used.

(e) Selective Amplification of Hybridomas by Selective Culture Medium

On the day following the start of culturing mentioned in the above section (d) (Day 1), 2 drops (approx. 0.1 ml) HAT culture medium (100 μM hypoxanthine, 0.4 μM aminopterin and 16 μM thymidine added to NS-1 culture medium) were added to the cells with a Pasteur pipette. On Days 2, 3, 5 and 8, half of each culture medium (approx. 0.1 ml) was replaced with fresh HAT medium, and on Day 11, half of each culture medium was replaced with fresh HT culture medium (HAT culture medium not containing aminopterin). On Day 14, for all the wells in which hybridoma growth was observed to the naked eye, positive wells were investigated by enzyme-linked immunoadsorbent assay (ELISA). Specifically, the polystyrene 96-well plate was respectively coated with polypeptides A, B and C serving as antigens, washed using PBS for washing (containing 0.05% Tween 20), and unadsorbed peptides were thus removed. In addition, the uncoated portion of each well was blocked with 1% BSA. 0.1 ml of supernatant from wells in which hybridoma growth was confirmed was added to each polypeptide-coated well, and the plate was stood at room temperature for approximately 1 hour.

Horseradish peroxidase-labeled goat anti-mouse immunoglobulin was added as a secondary antibody, and the plate was again stood at room temperature for approximately another 1 hour. A substrate of hydrogen peroxide and o-

phenylenediamine was added, and the degree of color development was measured as absorbance at 492 nm using a microplate light absorbency measuring device (MRP-A4, Tosoh).

(f) Hybridoma Cloning

Hybridomas in wells positive with respect to individual antigen peptides, as obtained in the foregoing section (e), were monocloned according to the limiting dilution method. Specifically, hybridomas were diluted to 5, 1 and 0.5 per well and were respectively added to 36, 36 and 24 wells of a 96 microwells. On Day 5 and Day 12, approximately 0.1 ml NS-1 medium was added to each well. Approximately 2 weeks after cloning began, the ELISA noted in section (e) was performed for groups in which sufficient hybridoma growth was visually confirmed and 50% or more wells were negative for colony formation. If all tested wells were not positive, 4 to 6 antibody-positive wells in which the number of colonies was 1 were selected, and recloning was performed. Finally, as shown in Table 1 and Table 2, 12, 20 and 9 hybridomas were obtained which produced monoclonal antibodies against polypeptide A, polypeptide B or polypeptide C, respectively.

(g) Hybridoma Culturing and Monoclonal Antibody Purification

Each obtained hybridoma was cultured in NS-1 medium and a 10 to 100 μg/ml concentration of monoclonal antibody was successfully obtained from the supernatant thereof. In addition, BALB/c mice given an one week prior intraperitoneal administration of pristane were given a similar intraperitoneal administration of $1x10^7$ cells of obtained hybridomas, and after 1 to 2 weeks, abdominal fluid containing 4 to 7 mg/ml of monoclonal antibody was successfully obtained. The abdominal fluid obtained was salted out by 40% saturated ammonium sulfate, and IgG class antibodies were adsorbed to Protein A Affigel (Bio-Rad) and purified by elution with a 0.1M citric acid buffer, pH 5.

(h) Determination of Monoclonal Antibody Class and Subclass

In accordance with the above-discussed ELISA, the supernatant of monoclones obtained in section (f) were added to microtitration plates respectively coated with polypeptide A, polypeptide B or polypeptide C. After washing with PBS, isotype-specific rabbit anti-mouse IgG antibodies (Zymed Lab.) were added. After washing with PBS, horseradish peroxidase-labeled goat anti-rabbit IgG (H+L) was added, and class and subclass were determined using hydrogen peroxide and 2.2'-azino-di(3-ethylbenzthiazolinic acid) as a substrate.

(i) Specificity of Anti-MT-MMP Monoclonal Antibodies

The cross-reactivity of five varieties of anti-MT-MMP monoclonal antibodies (monoclone numbers 113-5B7, 113-15E7, 114-1F1, 114-2F2 and 118-3B1) exhibiting a positive reaction against a human MT-MMP peptide was determined by the ELISA noted in the foregoing section (e), using as respective antigens: proMMP-1 (Clin. Chim. Acta, 219:1-14, 1993), proMMP-2 (Clin. Chim. Acta, 221:91-103, 1993) and proMMP-3 (Clin. Chim. Acta, 211:59-72, 1992) respectively purified from the supernatant of nomal human skin fibroblast (NB1RGB) culture; proMMP-7 purified from the supernatant of human rectal carcinoma cell (CaR-1) culture (Cancer Res., 50:7758-7764, 1990), proMMP-8 purified from human neutrophils (Biol. Chem. Hoppe-Seyler, 371 supp:295-304, 1990) and proMMP-9 purified from the supernatant of human fibrosarcoma cells (HT1080) culture (J. Biol. Chem., 267: 21712-21719, 1992).

Specifically, using a polystyrene 96-well plate, each well was coated by adding 50 ng/well of purified MMP-1, MMP-2, MMP-3, MMP-7, MMP-8 and MMP-9, respectively. Washing was performed with PBS for washing and non-adsorbed antigen was removed, and the uncoated portion of each well was blocked with PBS containing 3% skim milk. 1 μg/well of each MT-MMP monoclonal antibody was respectively added to each well and stood at room temperature for approximately 1 hour. After washing plate, peroxidase-labeled goat anti-mouse immunoglobulin was added as a secondary antibody, and the plate was again stood at room temperature for approximately 1 hour. A substrate of hydrogen peroxide and o-phenylene

diamine was added, and the degree of color development was measured absorbance at 492 nm using a microplate light absorbency measuring device (MRP-A4, Tosoh).

In results, as shown in Table 3, each anti-MT-MMP monoclonal antibody showed no reactivity against purified MMPs other than the MT-MMP supplied for testing.

TABLE 1

Polypeptide	Monoclone No.	Subclass/Chain
A	114-1F2	γ1/κ
	114-2F2	γ1/κ
	114-3H7	γ1/κ
	114-5E4	γ1/κ
	114-6G6	γ1/κ
	114-8D10	γ1/κ
	114-9H3	μ/κ
	114-15E8	γ1/κ
	114-16C11	γ1/κ
	114-18E4	γ1/κ
	114-19F11	γ1/κ
	114-20H5	μ/κ
B	113-1E3	γ3/κ
	113-2E9	γ3/κ
	113-3F6	γ2b/κ
	113-4H7	γ3/κ
	113-5B7	γ3/κ
	113-7C6	γ2b/κ
	113-9G9	γ3/κ
	113-10F2	γ3/κ
	113-13G11	γ3/κ
	113-15E7	γ3/κ
	113-16H8	γ3/κ
	113-17G12	μ/κ
	113-19A10	μ/κ
	113-20G11	γ3/κ
	113-21H3	γ1/κ
	113-26D3	μ/κ
	113-44C1	γ1/κ
	113-46B7	γ1/κ
	113-53G5	μ/κ
	113-63E8	γ1/κ

TABLE 2

Polypeptide	Monoclone No.	Subclass/Chain
C	118-3B1	γ2b/κ
	118-6F3	γ2b/κ
	118-8D11	γ1/κ
	118-9B11	γ1/κ
	118-13D11	α/κ
	118-18C12	γ1/κ
	118-20A3	γ2b/κ
	118-25C3	γ1/κ
	118-26F5	γ3/κ

TABLE 3

Monoclone No.	Cross reactivity					
	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9
113-5B7	-	-	-	-	-	-
113-15E7	-	-	-	-	-	-
114-1F2	-	-	-	-	-	-
114-2F2	-	-	-	-	-	-
118-3B1	-	-	-	-	-	-

- :No reaction

Working Example 4 Expression and Identification of Gene Product

By means of EcoR I cleavage, an insertion fragment was excised from the recombinant pBluescript containing a cloned MT-MMP gene, constructed in section (f) of Working Example 1. Cloning was then carried out at an EcoR I site

of the eukaryotic expression vector pSG5 (Stratagene). Then, human fibrosarcoma cells (HT1080) were transfected with said recombinant pSG5 by a calcium phosphate method. Specifically, 20 μg of recombinant pSG5 and 62 μl of 2M $CaCl_2$ was added to distilled water, and 2x HBSP solution (50mM HEPES buffer, pH 7.1 containing 1.5mM Na_2HPO_4, 10mM KCl, 280mM NaCl and 12mM glucose) was added to the bottom of the tube to form a total volume of 1 ml. This material was mixed, stood at room temperature for approximately 30 minutes, and thorough precipitate formation was carried out. The precipitate was dispersed by pipetting, added dropwise to HT1080 cells and incubated for approximately 4 hours in a CO_2 incubator. Next, the culture medium was removed, a 15% glycerol solution was added and treated for 1 to 3 hours, the glycerol was discarded by suction, washed with PBS and fresh culture medium containing ^{35}S-methionine was added. Culturing was continued, and cellular proteins were labeled by ^{35}S. Incidentally, expression of MT-MMP genes in HT1080 cells cannot be detected by Northern Blot analysis.

The cells were incubated for 1 hour at 4°C in a lysing buffer solution (0.01M Tris-HCl buffer, pH 8 containing 1% Triton X-100, 1% bovine hemoglobin, 1mM iodoacetamide, 0.2U trypsin inhibitor, 1mM PMSF and 0.14M NaCl). The cell lysate was centrifuged and the supernatant was recovered. Sepharose-4B (Pharmacia) coupled with a monoclonal antibody obtained in Working Example 3 was added to the supernatant, the material was incubated at 4°C for 2 hours with

agitation, and immunoprecipitation was carried out. Monoclonal antibodies against polypeptide A used in immunoprecipitation were two of the 12 obtained in Working Example 3 which had low non-specific reactivity (monoclone numbers 114-1F2 and 114-2F2 [Assignment No. FERM BP-4743]). Next, Sepharose 4B coupled with monoclonal antibodies subjected to immunoprecipitation were precipitated by centrifugation, washed three times with a washing solution (0.01M Tris-HCl buffer, pH 8 containing 1% Triton X-100, 1% bovine hemoglobin and 0.14M NaCl), and lastly, washed with 0.05M Tris-HCl buffer, pH 6.8. A sample buffer for SDS polyacrylamide electrophoresis was added to washed Sepharose-4B coupled with a monoclonal antibody, boiled 5 minutes at 100°C, and SDS polyacrylamide electrophoresis was carried out. The electrophoresed gel was layered with X-ray film (Kodak XR), 1 week autoradiography was then carried out at -70°C, and the developed X-ray films were traced by a densitometer to measure signal intensity. With each of the anti-MT-MMP monoclonal antibodies used (monoclone numbers 114-1F2 and 114-2F2), the immunoprecipitate contained a 63 kDa protein. In cells transfected with a pSG5 vector alone not containing an MT-MMP gene as a control, anti-MT-MMP monoclonal antibodies (monoclone numbers 114-1F2 and 114-2F2) did not precipitate a 63 kDa protein. The 63 kDa molecular weight of the protein detected by immunoprecipitation nearly matched a molecular weight of 65.78 kDa calculated from the amino acid sequence denoted by

Sequence Sheet sequence number 1. In addition, a variant MT-MMP expression plasmid was constructed in which amino acids from position 13 to position 101 were deleted from the amino acid sequence denoted by Sequence Sheet sequence number 1, HT1080 cells was transfected with said variant as stated above, and immunoprecipitation was carried out. With HT1080 cells to which the variant MT-MMP gene was introduced, a 63 kDa protein was not detected, and a 55 kDa protein was detected. This molecular weight matched a molecular weight predicted from the introduced deletion.

EXPERIMENTAL EXAMPLE

(a) Activation of proMMP-2 by MT-MMP Expression

Recombinant pSG5 carrying a cloned MT-MMP gene, constructed in Working Example 4, and a pSG5 vector alone, serving as a control, similarly transfected into HT1080 cells by the calcium phosphate method mentioned in Working Example 4, or into mouse embryonic fibroblasts NIH3T3. However, a regular fresh culture medium was used in lieu of the fresh culture medium containing ^{35}S-methionine. Both the HT1080 cells and the NIH3T3 cells secreted proMMP-2 and proMMP-9 (corresponding respectively to the 66 kDa and 97.4 kDa bands in Figure 6), and in cells transfected with an MT-MMP gene, MT-MMP expression was confirmed by immunoprecipitation experiments (See Working Example 4).

The transfectants obtained were cultured for 24 hours in a serum free medium and the recovered culture supernatant

was supplied for zymography. The culture supernatant was mixed with an SDS polyacrylamide electrophoresis buffer (non-reducing condition) and left at 4°C overnight. Electrophoresis was then performed at 4°C, with a 20 mA current, using a 10% polyacrylamide gel containing 1 mg/ml casein. After electrophoresis, the gel was washed with a gelatinase-buffer (Tris-HCl buffer, pH 7.6 containing 5mM $CaCl_2$ and 1 μM $ZnSO_4$) containing 2.5% Triton X-100 with gentle agitation for 15 minutes, and this operation was repeated twice. Next, the gel was immersed in a gelatinase-buffer containing 1% Triton X-100 and stood at 37°C overnight. The buffer was discarded and the gel was stained for 1 hour with 0.02% Coomassie Brilliant Blue-R (dissolved in 50% methanol/10% acetic acid) and destained by immersion in a destaining solution (5% methanol, 7.5% acetic acid).

As shown in Figure 6, MT-MMP gene-transfected HT1080 cells produced new 64 kDa and 62 kDa bands, confirming proMMP-2 activation. This active-form MMP-2 exhibited the same molecular weight as an active-form MMP-2 molecule induced by treatment of cells with 100 μg/ml of concanavalin A and reacted specifically against anti-MMP-2 monoclonal antibodies. This activation was not observed in a control transfected with a vector alone. Likewise, proMMP-9 showed no change in molecular weight and no activation similar to that observed in control cells. Such activation of proMMP-2 depending on MT-MMP expression was also observed in MT-MMP gene-transfected NIH3T3 cells.

(b) Activation of ProMMP-2 by MT-MMP Expression Cell Membrane Fraction

In a manner similar to that noted in the above section (a), African green monkey kidney-derived COS-1 cells were transfected with recombinant pSG5 containing cloned MT-MMP gene, or with control pSG5 vector alone by a calcium phosphate method. A cell membrane fraction was then prepared from the obtained transfectant according to the method of Strongin et al. (J. Biol. Chem., 268:14033-14039, 1993).

The transfectant was washed with PBS, and cells were harvested by centrifugation and suspended in a 25mM Tris-HCl buffer, pH 7.4 containing 8.5% sucrose, 50mM NaCl, 10mM N-ethylmaleimide, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin and 1mM phenylmethylsulfonyl fluoride. The cell suspension was homogenized in a Dounce homogenizer, and the homogenate was centrifuged (3000x g, 10 min., 4°C). The resulting supernatant was ultracentrifuged (100,000x g, 2 hours) and the precipitate was suspended in a 25mM Tris-HCl buffer, pH 7.4 containing 50mM NaCl, 10mM N-ethylmaleimide, 10 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin and 1mM phenylmethylsulfonyl fluoride. This suspension was fractionated by discontinuous sucrose density gradient centrifugation (20, 30, 50, 60% sucrose solutions; 100,000x g; 2 hours; 4°C), and bands of cell membrane fractions appeared were recovered. These fractions

were precipitated again by ultracentrifugation (100,000x g, 2 hours), suspended in 25mM HEPES/KOH buffer, pH 7.5 containing 0.1mM $CaCl_2$ and 0.25% Triton X-100, and adjusted to a final protein concentration of 1-2 mg/ml. This suspension was ultracentrifuged (100,000x g, 1.5 hours, 4°C) to remove insoluble residue, and the supernatant obtained was taken as a cell membrane fraction.

Cell membrane fractions (protein content 20 μg) respectively prepared from untreated COS-1 cells or from COS-1 cells transfected with pSG5 vector alone or pSG5 vector with an MT-MMP gene were incubated with HT1080 cell culture supernatant at 37°C for 2 hours. Using these samples, the zymography noted in the above section (a) was performed.

In the results, new 64 kDa and 62 kDa bands appeared and the activation of proMMP-2 present in HT1080 cell culture supernatant was observed only when cell membrane fractions derived from MT-MMP gene-transfected COS-1 cells were used (see Figure 7), and the activation of proMMP-2 was inhibited by the addition of recombinant (r) human TIMP-2. These results exhibited the activation of proMMP-2 by MT-MMP expressed on a cell membrane.

(c) Stimulation of cellular invasion *in vitro* due to MT-MMP expression

Invasion of cells was assayed by modified Boyden Chamber method (Cancer Res., 47:3239-3245, 1987), and

operations were carried out in accordance with the manufucture's instructions for a Biocoat Matrigel Invasion Chamber (Becton Dickinson).

In a manner similar to that noted in the foregoing section (a), HT1080 cells or NIH3T3 cells were transfected with recombinant pSG5 carrying a cloned MT-MMP gene, or a control pSG5 vector alone, by a calcium phosphate method, and each of these host cells secreted proMMP-2. The resulting transfectants were then suspended in DMEM medium containing 0.1% BSA, and 2×10^5 cells were seeded onto an uncoated filter (pore size 8 μm) or a preswelled Matrigel Coat filter in a Biocoat Matrigel Invasion Chambers. After 24 hours incubation in a CO_2 incubator at 37°C, the filters were fixed by 10 seconds immersion in methanol. The filters were then stained by hematoxylin for 3 minutes, washed, and stained by eosin for 10 seconds, and the number of cells invaded the bottom surface of the filters were counted under a light microscope (at a magnification of x 400).

In the MT-MMP gene-transfected HT1080 cells and NIH3T3 cells, more than twice as many invading cells were seen compared to cells transfected with the control vector alone (See Figure 8 Matrigel). Specifically, MT-MMP expression was seen to stimulate cellular invasion. Furthermore, the addition of 10 μg/ml of r-human TIMP-2 to this assay system clearly suppressed cellular invasion (see Figure 8 Matrigel+r-human TIMP-2).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows hydrophilic and hydrophobic distribution diagrams for the amino acid sequence of MT-MMP, according to the Kyte-Doolittle method.

Figures 2A, 2B, 2C, 2D, 2E, 2F, 2G and 2H are figures comparing sequential homology between the amino acid sequences of MT-MMP and those of the known MMP family (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10 and MMP-11). Letters in each figure indicate respective amino acids; A corresponding to Ala, C to Cys, D to Asp, E to Glu, F to Phe, G to Gly, H to His, I to Ile, K to Lys, L to Leu, M to Met, N to Asn, P to Pro, Q to Gln, R to Arg, S to Ser, T to Thr, V to Val, W to Trp and Y to Tyr. Figures 2A through 2H are an integral unit and comprise a single figure.

Figure 3 shows a relative expression of MT-MMP mRNA in various human tissues, according to Northern blot analysis.

Figure 4 shows a relative expression of MT-MMP mRNA in a normal tissue and a tumour tissue of two samples of human lung squamous cell carcinoma, according to Northern blot analysis.

Figure 5 shows results for detection, by immunoprecipitation, of MT-MMP proteins expressed in HT1080 cells transfected with MT-MMP cDNA. The figure shows a scan by a densitometer, and the darkened areas indicate the location of MT-MMP immunoprecipitated by anti-MT-MMP monoclonal antibody.

Figure 6 shows an activation of proMMP-2 by expression of MT-MMP, according to zymography of culture supernatant from HT1080 and NIH3T3 cells transfected with MT-MMP cDNA.

Figure 7 shows an activation of proMMP-2 by a cell membrane fraction of COS-1 cells transfected with MT-MMP cDNA, according to zymography.

Figure 8 shows a stimulation of the cellular invasion by expression of MT-MMP, according to a partially modified Boyden chamber method.

[Sequence Sheet 1]

Sequence No.: 1

Length of sequence: 582

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Protein

Sequence

Met	Ser	Pro	Ala	Pro	Arg	Pro	Ser	Arg	Cys	Leu	Leu	Leu	Pro	Leu		
1				5				10						15		
Leu	Thr	Leu	Gly	Thr	Ala	Leu	Ala	Ser	Leu	Gly	Ser	Ala	Gln	Ser		
				20				25						30		
Ser	Ser	Phe	Ser	Pro	Glu	Ala	Trp	Leu	Gln	Gln	Tyr	Gly	Tyr	Leu		
				35				40						45		
Pro	Pro	Gly	Asp	Leu	Arg	Thr	His	Thr	Gln	Arg	Ser	Pro	Gln	Ser		
				50				55						60		
Leu	Ser	Ala	Ala	Ile	Ala	Ala	Met	Gln	Lys	Phe	Tyr	Gly	Leu	Gln		
				65				70						75		
Val	Thr	Gly	Lys	Ala	Asp	Ala	Asp	Thr	Met	Lys	Ala	Met	Arg	Arg		
				80				85						90		
Pro	Arg	Cys	Gly	Val	Pro	Asp	Lys	Phe	Gly	Ala	Glu	Ile	Lys	Ala		
				95				100						105		
Asn	Val	Arg	Arg	Lys	Arg	Tyr	Ala	Ile	Gln	Gly	Leu	Lys	Trp	Gln		
				110				115						120		
His	Asn	Glu	Ile	Thr	Phe	Cys	Ile	Gln	Asn	Tyr	Thr	Pro	Lys	Val		
				125				130						135		
Gly	Glu	Tyr	Ala	Thr	Tyr	Glu	Ala	Ile	Arg	Lys	Ala	Phe	Arg	Val		
				140				145						150		
Trp	Glu	Ser	Ala	Thr	Pro	Leu	Arg	Phe	Arg	Glu	Val	Pro	Tyr	Ala		
				155				160						165		

[Sequence Sheet 2]

Sequence No.: 1 (continued)

```
Tyr Ile Arg Glu Gly His Glu Lys Gln Ala Asp Ile Met Ile Phe
                170                 175                 180
Phe Ala Glu Gly Phe His Gly Asp Ser Thr Pro Phe Asp Gly Glu
                185                 190                 195
Gly Gly Phe Leu Ala His Ala Tyr Phe Pro Gly Pro Asn Ile Gly
                200                 205                 210
Gly Asp Thr His Phe Asp Ser Ala Glu Pro Trp Thr Val Arg Asn
                215                 220                 225
Glu Asp Leu Asn Gly Asn Asp Ile Phe Leu Val Ala Val His Glu
                230                 235                 240
Leu Gly His Ala Leu Gly Leu Glu His Ser Ser Asp Pro Ser Ala
                245                 250                 255
Ile Met Ala Pro Phe Tyr Gln Trp Met Asp Thr Glu Asn Phe Val
                260                 265                 270
Leu Pro Asp Asp Asp Arg Arg Gly Ile Gln Gln Leu Tyr Gly Gly
                275                 280                 285
Glu Ser Gly Phe Pro Thr Lys Met Pro Pro Gln Pro Arg Thr Thr
                290                 295                 300
Ser Arg Pro Ser Val Pro Asp Lys Pro Lys Asn Pro Thr Tyr Gly
                305                 310                 315
Pro Asn Ile Cys Asp Gly Asn Phe Asp Thr Val Ala Met Leu Arg
                320                 325                 330
Gly Glu Met Phe Val Phe Lys Lys Arg Trp Phe Trp Arg Val Arg
                335                 340                 345
Asn Asn Gln Val Met Asp Gly Tyr Pro Met Pro Ile Gly Gln Phe
                350                 355                 360
Trp Arg Gly Leu Pro Ala Ser Ile Asn Thr Ala Tyr Glu Arg Lys
                365                 370                 375
```

[Sequence Sheet 3]

Sequence No.: 1 (continued)

```
Asp Gly Lys Phe Val Phe Phe Lys Gly Asp Lys His Trp Val Phe
                380                 385                 390
Asp Glu Ala Ser Leu Glu Pro Gly Tyr Pro Lys His Ile Lys Glu
                395                 400                 405
Leu Gly Arg Gly Leu Pro Thr Asp Lys Ile Asp Ala Ala Leu Phe
                410                 415                 420
Trp Met Pro Asn Gly Lys Thr Tyr Phe Phe Arg Gly Asn Lys Tyr
                425                 430                 435
Tyr Arg Phe Asn Glu Glu Leu Arg Ala Val Asp Ser Glu Tyr Pro
                440                 445                 450
Lys Asn Ile Lys Val Trp Glu Gly Ile Pro Glu Ser Pro Arg Gly
                455                 460                 465
Ser Phe Met Gly Ser Asp Glu Val Phe Thr Tyr Phe Tyr Lys Gly
                470                 475                 480
Asn Lys Tyr Trp Lys Phe Asn Asn Gln Lys Leu Lys Val Glu Pro
                485                 490                 495
Gly Tyr Pro Lys Ser Ala Leu Arg Asp Trp Met Gly Cys Pro Ser
                500                 505                 510
Gly Gly Arg Pro Asp Glu Gly Thr Glu Glu Glu Thr Glu Val Ile
                515                 520                 525
Ile Ile Glu Val Asp Glu Glu Gly Gly Gly Ala Val Ser Ala Ala
                530                 535                 540
Ala Val Val Leu Pro Val Leu Leu Leu Leu Leu Val Leu Ala Val
                545                 550                 555
Gly Leu Ala Val Phe Phe Phe Arg Arg His Gly Thr Pro Arg Arg
                560                 565                 570
Leu Leu Tyr Lys Gln Arg Ser Leu Leu Asp Lys Val
                575                 580
```

[Sequence Sheet 4]

Sequence No.: 2

Length of sequence: 3403

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: cDNA to mRNA

Origin:

Species: Human

Tissue: Placenta

[Sequence Sheet 5]

Sequence No.: 2 (continued)

```
AGTTCAGTGCCTACC GAAGACAAAGGCGCC CCGAGGGAGTGGCGG TGCGACCCCAGGGCG      60

TGGGCCCGGCCGCGG AGCCACACTGCCCGG CTGACCCGGTGGTCT CGGACCATGTCTCCC     120
                                                        MetSerPro
                                                        1

GCCCCAAGACCCTCC CGTTGTCTCCTGCTC CCCCTGCTCACGCTC GGCACCGCGCTCGCC     180
AlaProArgProSer ArgCysLeuLeuLeu ProLeuLeuThrLeu GlyThrAlaLeuAla
5               10              15              20

TCCCTCGGCTCGGCC CAAAGCAGCAGCTTC AGCCCCGAAGCCTGG CTACAGCAATATGGC     240
SerLeuGlySerAla GlnSerSerSerPhe SerProGluAlaTrp LeuGlnGlnTyrGly
25              30              35              40

TACCTGCCTCCCGGG GACCTACGTACCCAC ACACAGCGCTCACCC CAGTCACTCTCAGCG     300
TyrLeuProProGly AspLeuArgThrHis ThrGlnArgSerPro GlnSerLeuSerAla
45              50              55              60

GCCATCGCTGCCATG CAGAAGTTTTACGGC TTGCAAGTAACAGGC AAAGCTGATGCAGAC     360
AlaIleAlaAlaMet GlnLysPheTyrGly LeuGlnValThrGly LysAlaAspAlaAsp
65              70              75              80

ACCATGAAGGCCATG AGGCGCCCCCGATGT GGTGTTCCAGACAAG TTTGGGGCTGAGATC     420
ThrMetLysAlaMet ArgArgProArgCys GlyValProAspLys PheGlyAlaGluIle
85              90              95              100

AAGGCCAATGTTCGA AGGAAGCGCTACGCC ATCCAGGGTCTCAAA TGGCAACATAATGAA     480
LysAlaAsnValArg ArgLysArgTyrAla IleGlnGlyLeuLys TrpGlnHisAsnGlu
105             110             115             120

ATTACTTTCTGCATC CAGAATTACACCCCC AAGGTGGGCGAGTAT GCCACATACGAGGCC     540
IleThrPheCysIle GlnAsnTyrThrPro LysValGlyGluTyr AlaThrTyrGluAla
125             130             135             140

ATTCGCAAGGCGTTC CGCGTGTGGGAGAGT GCCACACCACTGCGC TTCCGCGAGGTGCCC     600
IleArgLysAlaPhe ArgValTrpGluSer AlaThrProLeuArg PheArgGluValPro
145             150             155             160

TATGCCTACATCCGT GAGGGCCATGAGAAG CAGGCCGACATCATG ATCTTCTTTGCCGAG     660
TyrAlaTyrIleArg GluGlyHisGluLys GlnAlaAspIleMet IlePhePheAlaGlu
165             170             175             180
```

[Sequence Sheet 6]

Sequence No.: 2 (continued)

GGCTTCCATGGCGAC	AGCACGCCCTTCGAT	GGTGAGGGCGGCTTC	CTGGCCCATGCCTAC	720
GlyPheHisGlyAsp	SerThrProPheAsp	GlyGluGlyGlyPhe	LeuAlaHisAlaTyr	
185	190	195	200	
TTCCCAGGGCCCAAC	ATTGGAGGAGACACC	CACTTTGACTCTGCC	GAGCCTTGGACTGTC	780
PheProGlyProAsn	IleGlyGlyAspThr	HisPheAspSerAla	GluProTrpThrVal	
205	210	215	220	
AGGAATGAGGATCTG	AATGGAAATGACATC	TTCCTGGTGGCTGTG	CACGAGCTGGGCCAT	840
ArgAsnGluAspLeu	AsnGlyAsnAspIle	PheLeuValAlaVal	HisGluLeuGlyHis	
225	230	235	240	
GCCCTGGGGCTCGAG	CATTCCAGTGACCCC	TCGGCCATCATGGCA	CCCTTTTACCAGTGG	900
AlaLeuGlyLeuGlu	HisSerSerAspPro	SerAlaIleMetAla	ProPheTyrGlnTrp	
245	250	255	260	
ATGGACACGGAGAAT	TTTGTGCTTCCCGAT	GATGACCGCCGGGGC	ATCCAGCAACTTTAT	960
MetAspThrGluAsn	PheValLeuProAsp	AspAspArgArgGly	IleGlnGlnLeuTyr	
265	270	275	280	
GGGGGTGAGTCAGGG	TTCCCCACCAAGATG	CCCCCTCAACCCAGG	ACTACCTCCCGGCCT	1020
GlyGlyGluSerGly	PheProThrLysMet	ProProGlnProArg	ThrThrSerArgPro	
285	290	295	300	
TCTGTTCCTGATAAA	CCCAAAAACCCCACC	TATGGGCCCAACATC	TGTGACGGGAACTTT	1080
SerValProAspLys	ProLysAsnProThr	TyrGlyProAsnIle	CysAspGlyAsnPhe	
305	310	315	320	
GACACCGTGGCCATG	CTCCGAGGGGAGATG	TTTGTCTTCAAGAAG	CGCTGGTTCTGGCGG	1140
AspThrValAlaMet	LeuArgGlyGluMet	PheValPheLysLys	ArgTrpPheTrpArg	
325	330	335	340	
GTGAGGAATAACCAA	GTGATGGATGGATAC	CCAATGCCCATTGGC	CAGTTCTGGCGGGGC	1200
ValArgAsnAsnGln	ValMetAspGlyTyr	ProMetProIleGly	GlnPheTrpArgGly	
345	350	355	360	
CTGCCTGCGTCCATC	AACACTGCCTACGAG	AGGAAGGATGGCAAA	TTCGTCTTCTTCAAA	1260
LeuProAlaSerIle	AsnThrAlaTyrGlu	ArgLysAspGlyLys	PheValPhePheLys	
365	370	375	380	
GGAGACAAGCATTGG	GTGTTTGATGAGGCG	TCCCTGGAACCTGGC	TACCCCAAGCACATT	1320
GlyAspLysHisTrp	ValPheAspGluAla	SerLeuGluProGly	TyrProLysHisIle	
385	390	395	400	

[Sequence Sheet 7]

Sequence No.: 2 (continued)

```
AAGGAGCTGGGCCGA GGGCTGCCTACCGAC AAGATTGATGCTGCT CTCTTCTGGATGCCC   1380
LysGluLeuGlyArg GlyLeuProThrAsp LysIleAspAlaAla LeuPheTrpMetPro
   405             410             415             420

AATGGAAAGACCTAC TTCTTCCGTGGAAAC AAGTACTACCGTTTC AACGAAGAGCTCAGG   1440
AsnGlyLysThrTyr PhePheArgGlyAsn LysTyrTyrArgPhe AsnGluGluLeuArg
   425             430             435             440

GCAGTGGATAGCGAG TACCCCAAGAACATC AAAGTCTGGGAAGGG ATCCCTGAGTCTCCC   1500
AlaValAspSerGlu TyrProLysAsnIle LysValTrpGluGly IleProGluSerPro
   445             450             455             460

AGAGGGTCATTCATG GGCAGCGATGAAGTC TTCACTTACTTCTAC AAGGGGAACAAATAC   1560
ArgGlySerPheMet GlySerAspGluVal PheThrTyrPheTyr LysGlyAsnLysTyr
   465             470             475             480

TGGAAATTCAACAAC CAGAAGCTGAAGGTA GAACCGGGCTACCCC AAGTCAGCCCTGAGG   1620
TrpLysPheAsnAsn GlnLysLeuLysVal GluProGlyTyrPro LysSerAlaLeuArg
   485             490             495             500

GACTGGATGGGCTGC CCATCGGGAGGCCGG CCGGATGAGGGGACT GAGGAGGAGACGGAG   1680
AspTrpMetGlyCys ProSerGlyGlyArg ProAspGluGlyThr GluGluGluThrGlu
   505             510             515             520

GTGATCATCATTGAG GTGGACGAGGAGGGC GGCGGGGCGGTGAGC GCGGCTGCCGTGGTG   1740
ValIleIleIleGlu ValAspGluGluGly GlyGlyAlaValSer AlaAlaAlaValVal
   525             530             535             540

CTGCCCGTGCTGCTG CTGCTCCTGGTGCTG GCGGTGGGCCTTGCA GTCTTCTTCTTCAGA   1800
LeuProValLeuLeu LeuLeuLeuValLeu AlaValGlyLeuAla ValPhePhePheArg
   545             550             555             560

CGCCATGGGACCCCC AGGCGACTGCTCTAC TGCCAGCGTTCCCTG CTGGACAAGGTCTGA   1860
ArgHisGlyThrPro ArgArgLeuLeuTyr CysGlnArgSerLeu LeuAspLysVal...
   565             570             575             580

CGCCCATCCGCCGGC CCGCCCACTCCTACC ACAAGGACTTTGCCT CTGAAGGCCAGTGGC   1920


AGCAGGTGGTGGTGG GTGGGCTGCTCCCAT CGTCCCGAGCCCCCT CCCCGCAGCCTCCTT   1980
```

[Sequence Sheet 8]

Sequence No.: 2 (continued)

GCTTCTCTCTGTCCC CTGGCTGGCCTCCTT CACCCTGACCGCCTC CCTCCCTCCTGCCCC 2040

GGCATTGCATCTTCC CTAGATAGGTCCCCT GAGGGCTGAGTGGGA GGGCGGCCCTTTCCA 2100

GCCTCTGCCCCTCAG GGGAACCCTGTAGCT TTGTGTCTGTCCAGC CCCATCTGAATGTGT 2160

TGGGGGCTCTGCACT TGAAGGCAGGACCCT CAGACCTCGCTGGTA AAGGTCAAATGGGGT 2220

CATCTGCTCCTTTTC CATCCCCTGACATAC CTTAACCTCTGAACT CTGACCTCAGGAGGC 2280

TCTGGGGAACTCCAG GCCTGAAAGCCCCAG GTGTACCCAATTGGC AGCCTCTCACTACTC 2340

TTTCTGGCTAAAAGG AATCTAATCTTGTTG AGGGTAGAGACCCTG AGACAGTGTGAGGGG 2400

GTGGGGACTGCCAAG CCACCCTAAGACCTT GGGAGGAAAACTCAG AGAGGGTCTTCGTTG 2460

CTCAGTCAGTCAAGT TCCTCGGAGATCTTC CTCTGCCTCACCTAC CCCAGGGAACTTCCA 2520

AGGAAGGAGCCTGAG CCACTGGGGACTAAG TGGGCAGAAGAAACC CTTGGCAGCCCTGTG 2580

CCTCTCGAATGTTAG CCTTGGATGGGGCTT TCACAGTTAGAAGAG CTGAAACCAGGGGTG 2640

[Sequence Sheet 9]

Sequence No.: 2 (continued)

CAGCTGTCAGGTAGG GTGGGGCCGGTGGGA GAGGCCCGGGTCAGA GCCCTGGGGGTGAGC 2700

CTTAAGGCCACAGAG AAAGAACCTTGCCCA AACTCAGGCAGCTGG GGCTGAGGCCCAAAG 2760

GCAGAACAGCCAGAG GGGGCAGGAGGGGAC CAAAAAGGAAAATGA GGACGTGCAGCAGCA 2820

TTGGAAGGCTGGGGC CCGGCAGCCAGGTTA AAGCTAACAGGGGGC CATCAGGGTGGGCTT 2880

GTGGAGCTCTCAGGA AGGGCCCTGAGGAAG GCACACTTGCTCCTG TTGGTCCCTGTCCTT 2940

GCTGCCCAGGCAGGG TGGAGGGGAAGGGTA GGGCAGCCAGAGAAA GGAGCAGAGAAGGCA 3000

CACAAACGAGGAATG AGGGGCTTCACGAGA GGCCACAGGGCCTGG CTGGCCACGCTGTCC 3060

CGGCCTGCTCACCAT CTCAGTGAGGGACAG GAGCTGGGGCTGCTT AGGCTGGGTCCACGC 3120

TTCCCTGGTGCCAGC ACCCCTCAAGCCTGT CTCACCAGTGGCCTG CCCTCTCGCTCCCCC 3180

ACCCAGCCCACCCAT TGAAGTCTCCTTGGG TCCCAAAGGTGGGCA TGGTACCGGGGACTT 3240

GGGAGAGTGAGACCC AGTGGAGGGAGCAAG AGGAGAGGGATGTGG GGGGGTGGGGCACGG 3300

GTAGGGGAAATGGGG TGAACGGTGCTGGCA GTTCGGCTAGATTTC TGTCTTGTTTGTTTT 3360

TTTGTTTTGTTTAAT GTATATTTTTATTAT AATTATTATATAT

[Sequence Sheet 10]

Sequence No.: 3

Length of sequence: 7

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Pro Arg Cys Gly Val Pro Asp

1 5

[Sequence Sheet 11]

Sequence No.: 4

Length of sequence: 9

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Gly Asp Ala His Phe Asp Asp Asp Glu

1 5

[Sequence Sheet 12]

Sequence No.: 5

Length of sequence: 20

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: Other nucleic acid, synthetic DNA

Sequence

CC(C/A)(C/A)G(G/A/C)TG(T/C)(C/G)G(G/A/C)(G/A)(A/T)(G/C/T)CC

(T/A)GA

[Sequence Sheet 13]

Sequence No.: 6

Length of sequence: 25

Type of sequence: Nucleic acid

Number of chain: Double strand

Topology: Linear

Class of sequence: Other nucleic acid, synthetic DNA

Sequence

(T/C)TC(G/A)T(G/C)(G/A/C)TC(G/A)TC(G/A)AA(G/A)TG(G/A)(G/A)

(C/A/T)(G/A)TC(T/C)

[Sequence Sheet 14]

Sequence No.: 7

Length of sequence: 27

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Fragment type: Intermediate fragment

Sequence

Gly Gly Gly Ala Val Ser Ala Ala Ala Val
1 5 10
Val Leu Pro Val Leu Leu Leu Leu Leu Val
15 20
Leu Ala Val Gly Leu Ala Val Phe Phe Phe
25

[Sequence Sheet 15]

Sequence No.: 8

Length of sequence: 14

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Sequence

Arg Glu Val Pro Tyr Ala Tyr IIe Arg Glu
1 5 10
Gly His Glu Lys

[Sequence Sheet 16]

Sequence No.: 9

Length of sequence: 14

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Sequence

Asp Gly Asn Phe Asp Thr Val Ala Met Leu
1 5 10
Arg Gly Glu Met

[Sequence Sheet 17]

Sequence No.: 10

Length of sequence: 15

Type of sequence: Amino acid

Topology: Linear

Class of sequence: Peptide

Sequence

Pro Lys Ser Ala Leu Arg Asp Trp Met Gly
1 5 10
Cys Pro Ser Gly Gly
15

Claims

1. A native membrane-type matrix-metalloproteinase characterized by a continuous sequence of hydrophobic amino acids peculiar to membrane-binding proteins from amino acid number 533 to 562 in the C terminus domain shown in Sequence Sheet sequence number 1.

2. A native membrane-type matrix-metalloproteinase according to claim 1, characterized by the amino acid sequence from amino acid number 160 to 173, 320 to 333 and from 498 to 512 shown in Sequence Sheet sequence number 1.

3. A native membrane-type matrix-metalloproteinase according to claim 1, characterized by the amino acid sequence from amino acid number 1 to 173, 320 to 333, 498 to 512 and 563 to 582 shown in Sequence Sheet sequence number 1.

4. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2 which corresponds to the amino acid sequence of a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

5. A plasmid containing a DNA having the nucleotide sequence according to claim 4 and expressing a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

6. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence according to claim 4, and expressing a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

7. Monoclonal antibodies which peculiarly recognize a membrane-type matrix-metalloproteinase according to claim 1, 2 or 3.

8. A protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

9. A DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2 which encodes a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

10. A plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.

11. A host cell harbouring a plasmid containing a DNA having the nucleotide sequence shown in Sequence Sheet sequence number 2, and expressing the protein shown in Sequence Sheet sequence number 1.

12. Monoclonal antibodies which peculiarly recognize a protein having the amino acid sequence shown in Sequence Sheet sequence number 1.

Abstract

A novel metalloproteinase, DNA encoding therefor, a plasmid carrying said DNA sequence and a host cell harbouring said plasmid, and monoclonal antibodies peculiarly recognizing said protein.

Useful in applications pertaining to diagnosis of the presence of tumour cells, the degree of cancer malignancy, and other medical and physiological fields.

Fig. 1

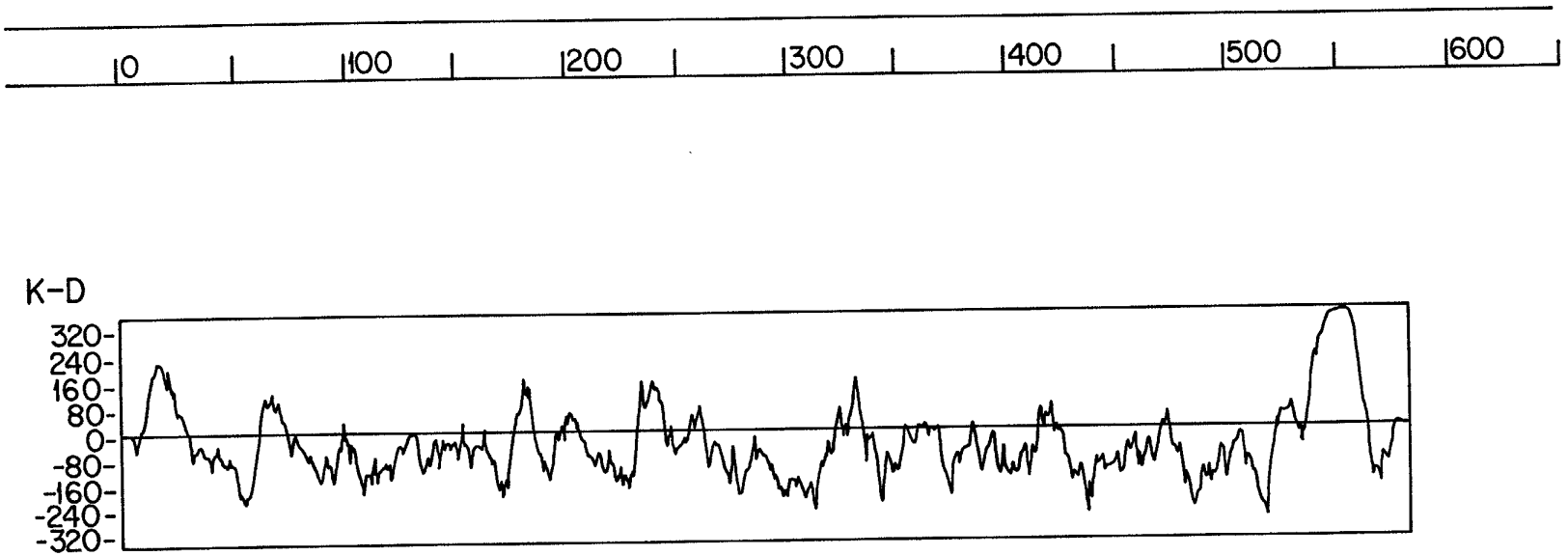


Fig. 2A

MMP-11	MAPAAWLRSA	AARALLPPML	LLLLQPPPL-	----------	------LARA	33
MMP-1	MHS-------	------FPPL	LLLLFWGVVS	HSFPATLETQ	EQDVDLVQKY	37
MMP-8	MFSLKTL---	-------PFL	LLLHVQISKA	FPVSSKEKNT	KTVQD----Y	36
MMP-10	MMHL------	-------AFL	VLLCLPVCSA	YPLSGAAKEE	DSNKDLAQQY	37
MMP-3	MKSL------	-------PIL	LLLCVAVCSA	YPLDGAARGE	DTSMNLVQKY	37
MMP-9	MSLWQP----	---LVLVLLV	LGCCFAAPRQ	RQSTLVLFPG	DLRTNLTDRQ	43
MMP-2	----------	----------	------AP--	--SPIIKFPG	DVAPK-TDKE	19
MMP-7	MR--------	---LTVLCAV	CLLPGSLALP	LPQEAGGMSE	------LQWE	33
MT-MMP	MSPAP-----	------RPSR	CLLLPLLTLG	TALASLGSAQ	SSSFS-PEAW	38
Consensus	M...------	------.P.L	LLL.....	....	.....-....	50

MMP-11	LPPDVHHL--	---HAERR-G	PQPWHAALPS	SP---APAPA	TQEAPRPASS	74
MMP-1	L-EKYYNLKN	DGRQVEKRRN	SGPVVEKLKQ	MQEFFGLKVT	GKPDAETLKV	86
MMP-8	L-EKFYQLPS	NQYQSTRKNG	TNVIVEKLKE	MQRFFGLNVT	GKPNEETLDM	85
MMP-10	L-EKYYNLEK	DVKQFRRK-D	SNLIVKKIQG	MQKFLGLEVT	GKLDTDTLEV	85
MMP-3	L-ENYYDLKK	DVKQFVRRKD	SGPVVKKIRE	MQKFLGLEVT	GKLDSDTLEV	86
MMP-9	LAEEYLYRYG	YTRVAEMRGE	SKSLGPALLL	LQKQLSLPET	GELDSATLKA	93
MMP-2	LAVQYLNTF-	YGCPKE-SCN	LFVLKDTLKK	MQKFFGLPQT	GDLDQNTIET	67
MMP-7	QAQDYLKRF-	YLYDSETK-N	ANSLEAKLKE	MQKFFGLPIT	GMLNSRVIEI	81
MT-MMP	L-QQYGYLPP	GDLRTHTQRS	PQSLSAAIAA	MQKFYGLQVT	GKADADTMKA	87
Consensus	L-E.Y..L..	.....E....	......KL..	MQKF.GL.VT	GKLD..TL..	100

Fig. 2B

MMP-11	LRPPRCGVPD	-PSDGLSARN	RQKRFVLSGG	RWEKTDLTYR	ILRFPWQLVQ	123
MMP-1	MKQPRCGVPD	-VAQ-FVL--	----TE-GNP	RWEQTHLTYR	IENYTPDLPR	127
MMP-8	MKKPRCGVPD	-SGG-FML--	----TP-GNP	KWERTNLTYR	IRNYTPQLSE	126
MMP-10	MRKPRCGVPD	-VGH-FSS--	----FP-GMP	KWRKTHLTYR	IVNYTPDLPR	126
MMP-3	MRKPRCGVPD	-VGH-FRT--	----FP-GIP	KWRKTHLTYR	IVNYTPDLPK	127
MMP-9	MRTPRCGVPD	-LGR-FQT--	----FE-GDL	KWHHHNITYW	IQNYSEDLPR	134
MMP-2	MRKPRCGNPD	-VAN-YNF--	----FP-RKP	KWDKNQITYR	IIGYTPDLDP	108
MMP-7	MQKPRCGVPD	-VAE-YSL--	----FP-NSP	KWTSKVVTYR	IVSYTRDLPH	122
MT-MMP	MRRPRCGVPD	KFGAEIKANV	RRKRYAIQGL	KWQHNEITFC	IQNYTPKVGE	137
Consensus	MRKPRCGVPD	-VG.-F..--	----FP-G.P	KW..T.LTYR	I.NYTPDLP.	150

MMP-11	EQVRQTMAEA	LKVWSDVTPL	TFTEV-----	---HEGRADI	MIDFARYWDG	165
MMP-1	ADVDHAIEKA	FQLWSNVTPL	TFTKV-----	---SEGQADI	MISFVRGDHR	169
MMP-8	AEVERAIKDA	FELWSVASPL	IFTRI-----	---SQGEADI	NIAFYQRDHG	168
MMP-10	DAVDSAIEKA	LKVWEEVTPL	TFSRL-----	---YEGEADI	MISFAVKEHG	168
MMP-3	DAVDSAVEKA	LKVWEEVTPL	TFSRL-----	---YEGEADI	MISFAVREHG	169
MMP-9	AVIDDAFARA	FALWSAVTPL	TFTRV-----	---YSRDADI	VIQFGVAEHG	176
MMP-2	ETVDDAFARA	FQVWSDVTPL	RFSRI-----	---HDGEADI	MINFGRWEHG	150
MMP-7	ITVDRLVSKA	LNMWGKEIPL	HFRKV-----	---VWGTADI	MIGFARGAHG	164
MT-MMP	YATYEAIRKA	FRVWESATPL	RFREVPYAYI	REGHEKQADI	MIFFAEGFHG	187
Consensus	..VD.A..KA	F.VWS.VTPL	TF.RV-----	---.EG-ADI	MI.FA...HG	200

Fig. 2C

MMP-11	DDLPFDGPGG	ILAHAFFPKT	HREGDVHFDY	DETWTIGDDQ	GTD-------	208
MMP-1	DNSPFDGPGG	NLAHAFQPGP	GIGGDAHFDE	DERWTNNFT-	EYN-------	211
MMP-8	DNSPFDGPNG	ILAHAFQPGQ	GIGGDAHFDA	EETWTNTSA-	NYN-------	210
MMP-10	DFYSFDGPGH	VLAHAYPPGP	GLYGDIHFDD	DEKWTEDAS-	GTN-------	210
MMP-3	DFYPFDGPGN	VLAHAYAPGP	GINGDAHFDD	DEQWTKDTT-	GTN-------	211
MMP-9	DGYPFDGKDG	LLAHAFPPGP	GIQGDAHFDD	DELWSLGKG-	VVVPTRFGNA	225
MMP-2	DGYPFDGKDG	LLAHAFAPGT	GVGGDSHFDD	DELWTLGEG-	QVVRVKYGNA	199
MMP-7	DSYPFDGPGN	TLAHAFAPGT	GLGGDAHFDE	DERWTDGSSL	GIN-------	207
MT-MMP	DSTPFDGEGG	FLAHAYFPGP	NIGGDTHFDS	AEPWTVRNE-	DLN-------	229
Consensus	D.YPFDGPGG	.LAHAF.PGP	GIGGDAHFD.	DE.WT....-	..N-------	250

MMP-11	----------	----------	----------	----------	----------	208
MMP-1	----------	----------	----------	----------	----------	211
MMP-8	----------	----------	----------	----------	----------	210
MMP-10	----------	----------	----------	----------	----------	210
MMP-3	----------	----------	----------	----------	----------	211
MMP-9	DGAACHFPFI	FEGRSYSACT	TDGRSDGLPW	CSTTANYDTD	DRFGFCPSER	275
MMP-2	DGEYCKFPFL	FNGKEYNSCT	DTGRSDGFLW	CSTTYNFEKD	GKYGFCPHEA	249
MMP-7	----------	----------	----------	----------	----------	207
MT-MMP	----------	----------	----------	----------	----------	229
Consensus	----------	----------	----------	----------	----------	300

Fig. 2D

MMP-11	----------	----------	----------	----------	----------	208
MMP-1	----------	----------	----------	----------	----------	211
MMP-8	----------	----------	----------	----------	----------	210
MMP-10	----------	----------	----------	----------	----------	210
MMP-3	----------	----------	----------	----------	----------	211
MMP-9	LYTRDGNADG	KPCQFPFIFQ	GQSYSACTTD	GRSDGYRWCA	TTANYDRDKL	325
MMP-2	LFTMGGNAEG	QPCKFPFRFQ	GTSYDSCTTE	GRTDGYRWCG	TTEDYDRDKK	299
MMP-7	----------	----------	----------	----------	----------	207
MT-MMP	----------	----------	----------	----------	----------	229
Consensus	----------	----------	----------	----------	----------	350

MMP-11	----------	----------	----------	----------	----------	208
MMP-1	----------	----------	----------	----------	----------	211
MMP-8	----------	----------	----------	----------	----------	210
MMP-10	----------	----------	----------	----------	----------	210
MMP-3	----------	----------	----------	----------	----------	211
MMP-9	FGFCPTRADS	TVMGGNSAGE	LCVFPFTFLG	KEYSTCTSEG	RGDGRLWCAT	375
MMP-2	YGFCPETAMS	TVGG-NSEGA	PCVFPFTFLG	NKYESCTSAG	RSDGKMWCAT	348
MMP-7	----------	----------	----------	----------	----------	207
MT-MMP	----------	----------	----------	----------	----------	229
Consensus	----------	----------	----------	----------	----------	400

Fig. 2E

MMP-11	----------	---------L	LQVAA-HEFG	HVLGLQHTTA	AKALMSAFY-	237
MMP-1	----------	---------L	HRVAA-HELG	HSLGLSHSTD	IGALMYPSY-	240
MMP-8	----------	---------L	FLVAA-HEFG	HSLGLAHSSD	PGALMYPNY-	239
MMP-10	----------	---------L	FLVAA-HELG	HSLGLFHSAN	TEALMYPLYN	240
MMP-3	----------	---------L	FLVAA-HEIG	HSLGLFHSAN	TEALMYPLYH	241
MMP-9	TSNFDSDKKW	GFCPDQGYSL	FLVAA-HEFG	HALGLDHSSV	PEALMYPMY-	423
MMP-2	TANYDDDRKW	GFCPDQGYSL	FLVAA-HEFG	HAMGLEHSQD	PGALMAPIY-	396
MMP-7	----------	----------	FLYAATHELG	HSLGMGHSSD	PNAVMYPTY-	236
MT-MMP	----------	------GNDI	FLVAV-HELG	HALGLEHSSD	PSAIMAPFY-	261
Consensus	----------	---------L	FLVAA-HE.G	HSLGL.HS.D	P.ALMYP.Y-	450

MMP-11	TF--RYPLSL	SPDDCRGVQH	LYG-------	----------	----------	258
MMP-1	TF--SGDVQL	AQDDIDGIQA	IYG-------	----------	----------	261
MMP-8	AFRETSNYSL	PQDDIDGIQA	IYG-------	----------	----------	262
MMP-10	SFTELAQFRL	SQDDVNGIQS	LYG-------	----------	----------	263
MMP-3	SLTDLTRFRL	SQDDINGIQS	LYG-------	----------	----------	264
MMP-9	RF--TEGPPL	HKDDVNGIRH	LYGPRPEPEP	RPPTTTTPQP	TAPPTVCPTG	471
MMP-2	TY--TKNFRL	SQDDIKGIQE	LYG-------	----------	----------	417
MMP-7	GNGDPQNFKL	SQDDIKGIQK	LYGKRSNSRK	K---------	----------	267
MT-MMP	QWMDTENFVL	PDDDRRGIQQ	LYGGESGFPT	KMPPQPRTTS	RPSVPDKPKN	311
Consensus	.F.....F.L	SQDDI.GIQ.	LYG-------	----------	----------	500

Fig. 2F

MMP-11	----------	-------QPW	PTVTSRTPAL	GPQAGIDTNE	IAPLEPDAPP	291
MMP-1	----------	----------	RSQNPVQP-I	GPQTP-----	-------KAC	278
MMP-8	----------	----------	---LSSNP-I	QPTGP---ST	P------KPC	279
MMP-10	----------	---------P	PPASTEEP-L	VPTKS---VP	S-GSEMPAKC	289
MMP-3	----------	---------P	PPDSPETP-L	VPTEP---VP	P-EPGTPANC	290
MMP-9	PPTVHPSERP	TAGPTGPPSA	GPTGPPTA-G	PSTAT---TV	PLSPVD-DAC	516
MMP-2	----------	----------	---ASPDI-D	LGTGP---TP	TLGPVTPEIC	440
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	PTYGPNICDG	NFDTVAMLRG	EMFVFKKRWF	WRVRNNQVMD	GYPMPIGQFW	361
Consensus	----------	---------.	.......P-.	.PT..---..	.-.......C	550

MMP-11	DACEASFDAV	STIR-GELFF	FKAGFVWRLR	GGQL-QPGYP	ALASRHWQGL	339
MMP-1	DS-KLTFDAI	TTIR-GEVMF	FKDRFYMR-T	NPFY-PEVEL	NFTSVFWPQL	324
MMP-8	DP-SLTFDAI	TTLR-GEILF	FKDRYFWR-R	HPQL-QRVEM	NFISLFWPSL	325
MMP-10	DP-ALSFDAI	STLR-GEYLF	FKDRYFWR-R	SHWN-PEPEF	HLISAFWPSL	335
MMP-3	DP-ALSFDAV	STLR-GEILI	FKDRHFWR-K	SLRK-LEPEL	HLISSFWPSL	336
MMP-9	NV-NI-FDAI	AEIG-NQLYL	FKDGKYWRFS	EGRGSRPQGP	FLIADKWPAL	563
MMP-2	KQ-DIVFDGI	AQIR-GEIFF	FKDRFIWRTV	TPRD-KPMGP	LLVATFWPEL	487
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	RGLPASINTA	YERKDGKFVF	FKGDKHWVFD	EASLEPGYPK	HIKELGRG-L	410
Consensus	D.-...FDAI	.T.R-GE..F	FKDR..WR-.	....-.....	.L.S.FWP.L	600

Fig. 2G

MMP-11	P-SPVDAAFE	-DAQGHIWFF	QGAQYWVYDG	EKPVLG---P	APL-TELGLV	383
MMP-1	P-NGLEAAYE	FADRDEVRFF	KGNKYWAVQG	QNVLHG--YP	KDIYSSFGFP	371
MMP-8	P-TGIQAAYE	DFDRDLIFLF	KGNQYWALSG	YDILQG--YP	KDI-SNYGFP	371
MMP-10	P-SYLDAAYE	VNSRDTVFIF	KGNEFWAIRG	NEVQAG--YP	RGI-HTLGFP	381
MMP-3	P-SGVDAAYE	VTSKDLVFIF	KGNQFWAIRG	NEVRAG--YP	RGI-HTLGFP	382
MMP-9	P-RKLDSVFE	EPLSKKLFFF	SGRQVWVYTG	ASV-LG---P	RRL-DKLGLG	607
MMP-2	P-EKIDAVYE	APQEEKAVFF	AGNEYWIYSA	STLERG--YP	KPL-TSLGLP	533
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	PTDKIDAALF	WMPNGKTYFF	RGNKYYRFNE	ELRAVDSEYP	KNIKVWEGIP	460
Consensus	P-...DAAYE	........FF	.GN.YW...G	.....G--YP	..I-..LG.P	650

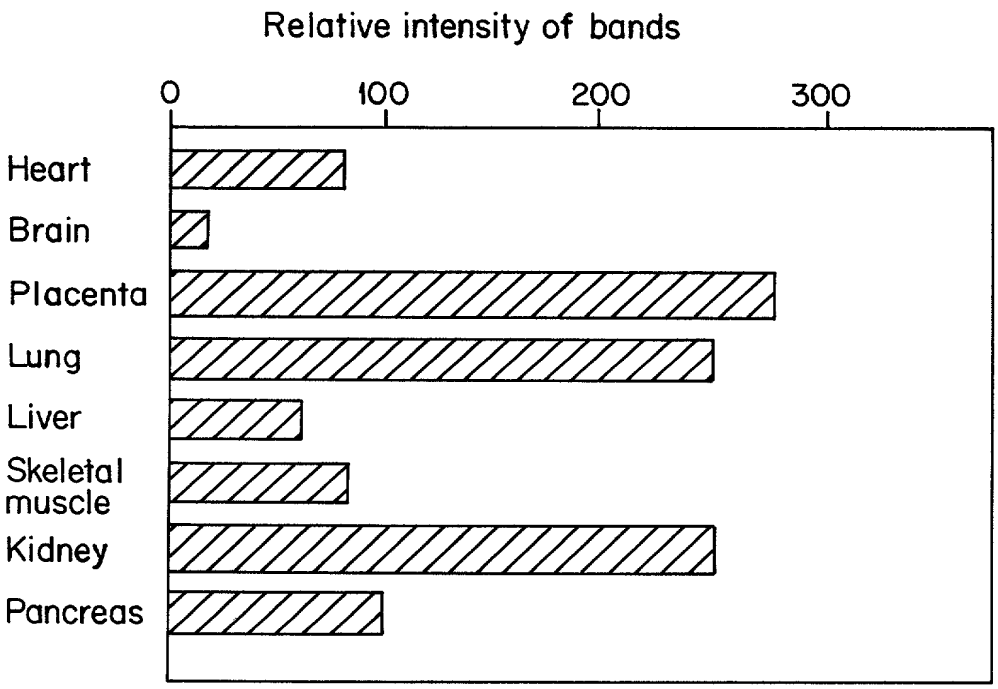
MMP-11	R--FPVHAAL	VWGPEKNKIY	FFRGRDYWRF	HPSTRRVDSP	VPRRATDWRG	431
MMP-1	RTVKHIDAAL	S-EENTGKTY	FFVANKYWRY	DEYKRSMDPG	YPKMIAHDFP	420
MMP-8	SSVQAIDAAV	F---YRSKTY	FFVNDQFWRY	DNQRQFMEPG	YPKSISGAFP	418
MMP-10	PTIRKIDAAV	S-DKEKKKTY	FFAADKYWRF	DENSQSMEQG	FPRLIADDFP	430
MMP-3	PTVRKIDAAI	S-DKEKNKTY	FFVEDKYWRF	DEKRNSMEPG	FPKQIAEDFP	431
MMP-9	ADVAQVTGAL	R-SGRGKM-L	LFSGRRLWRF	DVKAQMVDPR	SASEVDRMFP	655
MMP-2	PDVQRVDAAF	N-WSKNKKTY	IFAGDKFWRY	NEVKKKMDPG	FPKLIADAWN	582
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	ESPRGSFM-G	SDEVFTYFYK	GNKYWKFNNQ	KLKVEPGYPK	SALRDWMGCP	509
Consensus	..V...DAA.	.-.....KTY	FF...K.WR.	D.....M.PG	.P..I...FP	700

Fig. 2H

MMP-11	VPSE--IDAA	FQDADGYAYF	LRGRLYWKFD	PVKVKALEGF	PRLV------	473
MMP-1	GIGH--KVDA	V--FMKDGFF	-----YF-FH	GTRQYKFDPK	TKRILTLQ--	458
MMP-8	GIES--KVDA	V--FQQEHFF	-----HV-FS	GPRYYAFDLI	AQRVTRVA--	456
MMP-10	GVEP--KVDA	V--LQAFGFF	-----YF-FS	GSSQFEFDPN	ARMVTHIL--	468
MMP-3	GIDS--KIDA	V--FEEFGFF	-----YF-FT	GSSQLEFDPN	AKKVTHTL--	469
MMP-9	GVPL--DTHD	VFQYREKAYF	---CQDR-FY	WRVSSRSELN	QVDQVGYV--	697
MMP-2	AIPD--NLDA	VVDLQGGG--	-----HS-YF	FKGAYYLKLE	N-QSLKSVKF	621
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	SGGRPDEGTE	EETEVIIIEV	DEEGGGAVSA	AAVVLPVLLL	LLVLAVGLAV	559
Consensus	G...--..DA	V--.....F	-----..-F.	....	.........--	750

MMP-11	---GPD-FFG	CAE-----PA	NTFLX-----	----------	----------	489
MMP-1	---KANSWFN	CR------KN	----------	----------	----------	469
MMP-8	---RGNKWLN	CRY-----GX	----------	----------	----------	468
MMP-10	---KSNSWLH	C---------	----------	----------	----------	476
MMP-9	---TYD-ILQ	CPE-----DX	----------	----------	----------	477
MMP-2	GSIKSD-WLG	C---------	----------	----------	----------	708
MMP-7	----------	----------	----------	----------	----------	267
MT-MMP	FFFRRHGTPR	RLLYCQRSLL	DKV-------	----------	----------	582
Consensus	---....WL.	C.------..	----------	----------	----------	796

Fig. 3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200
300
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas

Fig. 4

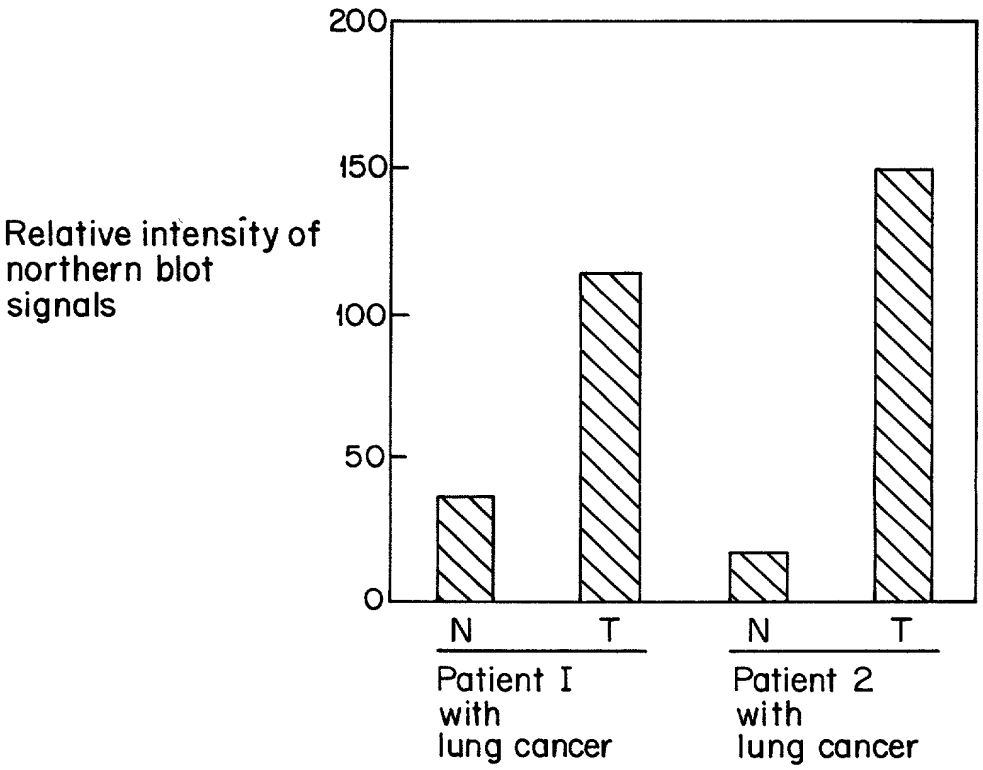
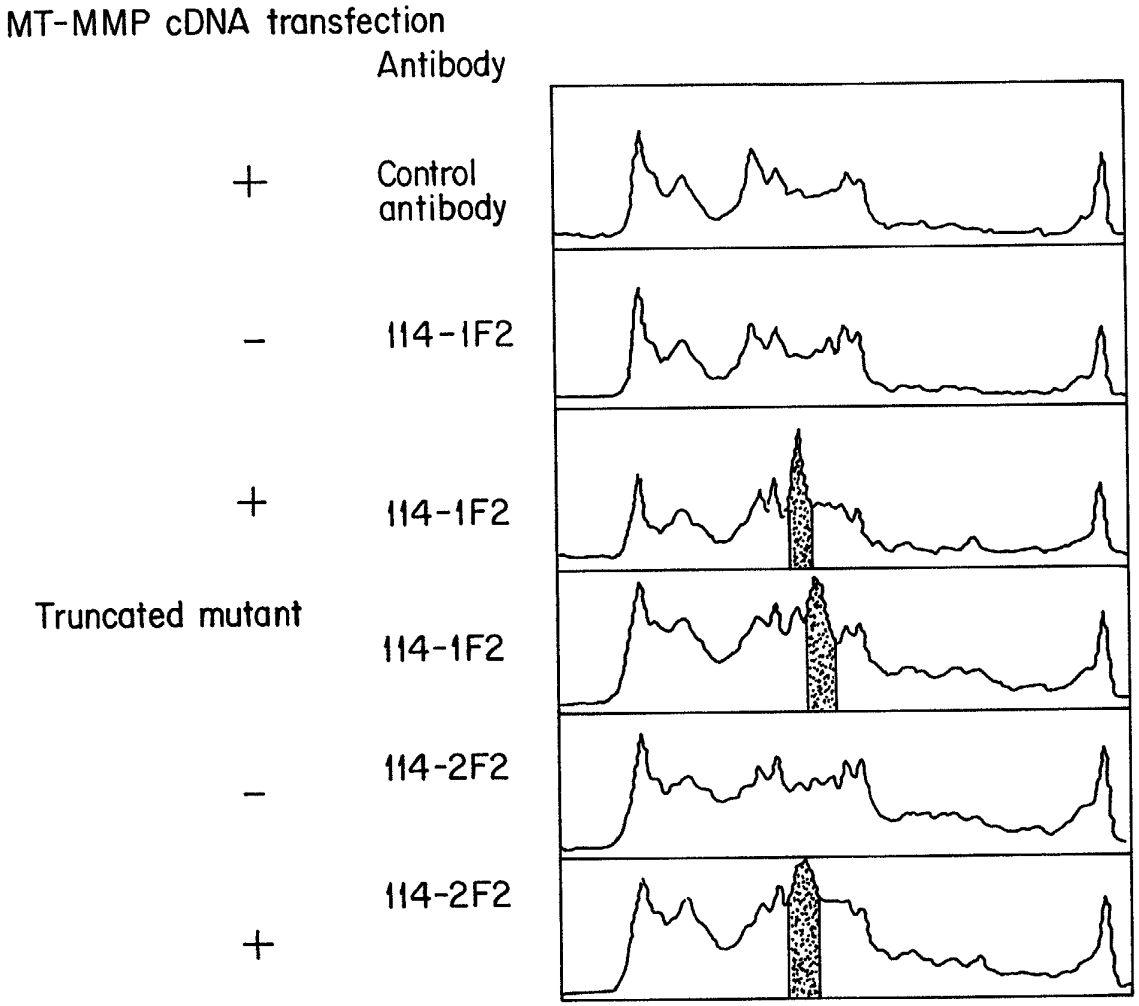

Relative intensity of northern blot signals
200
150
100
50
0
N
T
N
T
Patient I with lung cancer
Patient 2 with lung cancer

Fig. 5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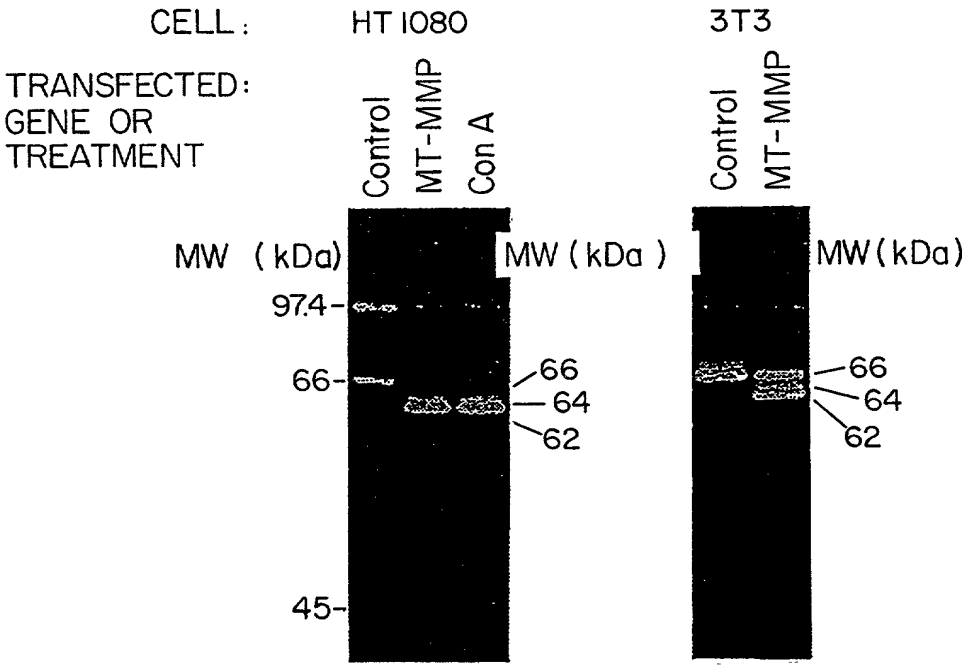
Antibody
+
Control antibody
−
114-1F2
+
114-1F2
Truncated mutant
114-1F2
−
114-2F2
+
114-2F2

Fig. 6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HT 1080
3T3
TRANSFECTED:
GENE OR
TREATMENT
Control
MT-MMP
Con A
Control
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MW (kDa)
MW (kDa)
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66
45
66
64
62
66
64
62

Fig. 7

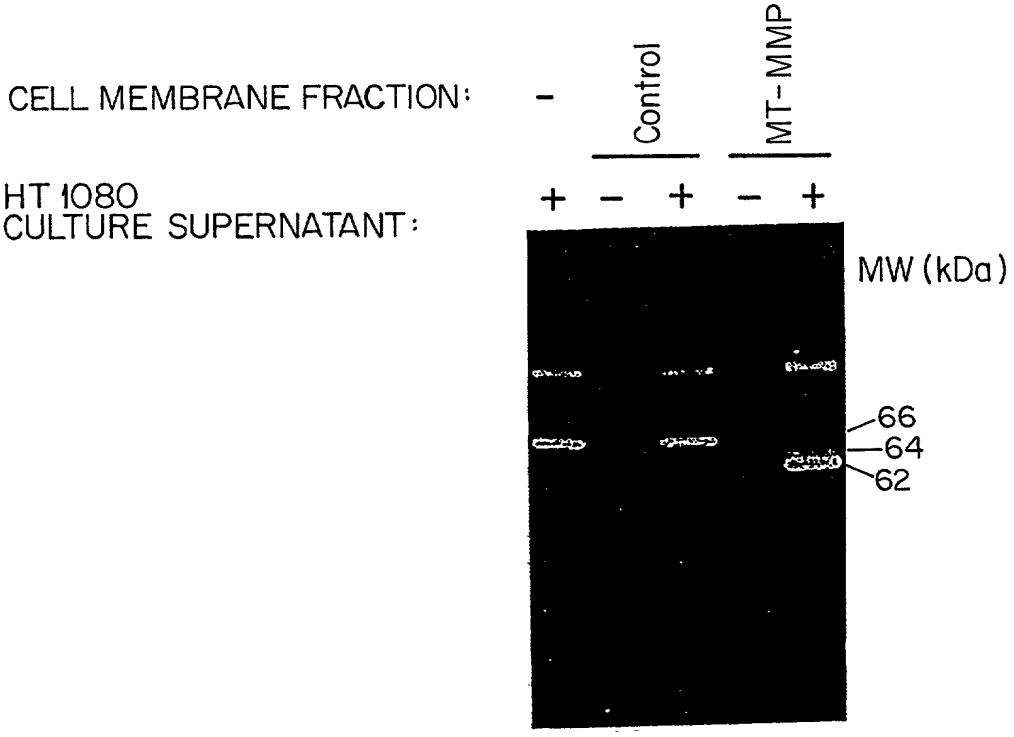
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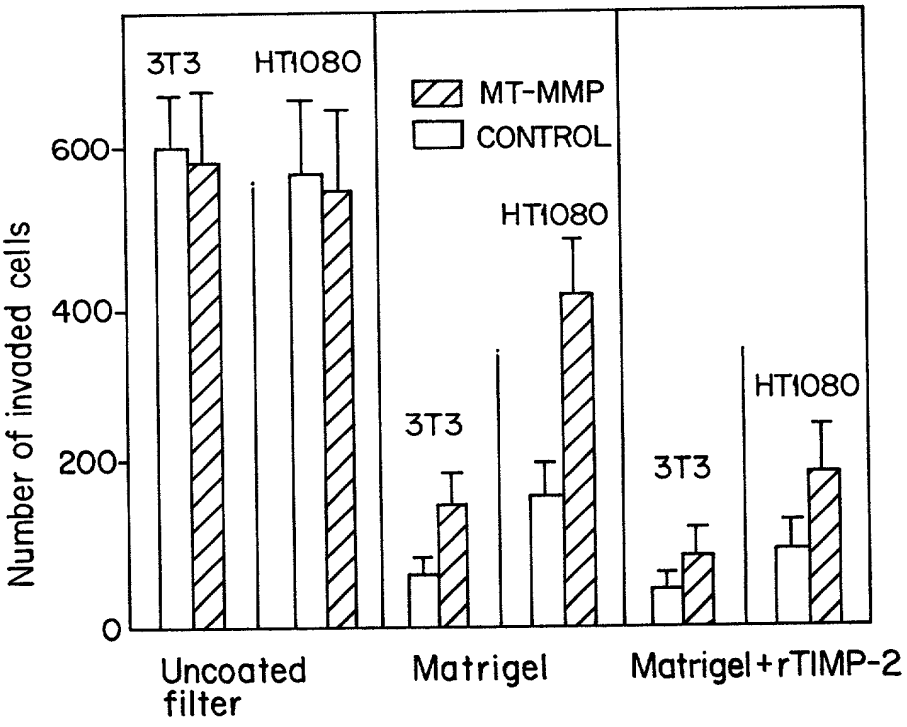
Fig. 8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400
200
0
Number of invaded cells
HT1080
3T3
HT1080
3T3
Uncoated filter
Matrigel
Matrigel+rTIMP-2

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO. 55-290P

As a below named inventor, I hereby declare that: my residence post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: * NOVEL METALLOPROTEINASE AND ENCODING DNA THEREFOR

Insert Title

Check Box If Appropriate — For Use Without Specification Attached

the specification of which is attached hereto unless one of the following boxes is checked:

☐ The Specification was filed on _____ and was assigned Serial No. _____ and was amended on _____

☐ was filed as PCT international application number _____ on _____ and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows:

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below:

Insert Priority Information (if appropriate)

Prior Foreign Application(s) (Number)	(Country)	(Month/Day/Year Filed)	Priority Claimed
Hei. 5-341061	Japan	11. 30. 1993	☒ Yes ☐ No
PCT/JP94/02009	Japan	11. 30. 1994	☒ Yes ☐ No
Hei. 7-109884	Japan	3. 31. 1995	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Country	Application No.	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status — patented, pending, abandoned)

*NOTE: Must be completed.

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

*Note: Must be completed — date this document is signed.

(USPTO Approved 3-90)
(Revised 7-93)

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<120> NOVEL METALLOPROTEINASE AND ENCODING DNA THEREFOR

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Phe Ser Pro Glu Ala Trp Leu Gln Gln Tyr Gly Tyr Leu Pro Pro Gly
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Asp Leu Arg Thr His Thr Gln Arg Ser Pro Gln Ser Leu Ser Ala Ala
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Ile Ala Ala Met Gln Lys Phe Tyr Gly Leu Gln Val Thr Gly Lys Ala
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Asp Ala Asp Thr Met Lys Ala Met Arg Arg Pro Arg Cys Gly Val Pro
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Asp Lys Phe Gly Ala Glu Ile Lys Ala Asn Val Arg Arg Lys Arg Tyr
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Asp Gly Glu Gly Gly Phe Leu Ala His Ala Tyr Phe Pro Gly Pro Asn
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Ile Gly Gly Asp Thr His Phe Asp Ser Ala Glu Pro Trp Thr Val Arg
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305                 310                 315                 320

Gly Asn Phe Asp Thr Val Ala Met Leu Arg Gly Glu Met Phe Val Phe
                325                 330                 335

Lys Lys Arg Trp Phe Trp Arg Val Arg Asn Asn Gln Val Met Asp Gly
            340                 345                 350

Tyr Pro Met Pro Ile Gly Gln Phe Trp Arg Gly Leu Pro Ala Ser Ile
        355                 360                 365

Asn Thr Ala Tyr Glu Arg Lys Asp Gly Lys Phe Val Phe Phe Lys Gly
    370                 375                 380

Asp Lys His Trp Val Phe Asp Glu Ala Ser Leu Glu Pro Gly Tyr Pro
```

385 390 395 400

Lys His Ile Lys Glu Leu Gly Arg Gly Leu Pro Thr Asp Lys Ile Asp
405 410 415

Ala Ala Leu Phe Trp Met Pro Asn Gly Lys Thr Tyr Phe Phe Arg Gly
420 425 430

Asn Lys Tyr Tyr Arg Phe Asn Glu Glu Leu Arg Ala Val Asp Ser Glu
435 440 445

Tyr Pro Lys Asn Ile Lys Val Trp Glu Gly Ile Pro Glu Ser Pro Arg
450 455 460

Gly Ser Phe Met Gly Ser Asp Glu Val Phe Thr Tyr Phe Tyr Lys Gly
465 470 475 480

Asn Lys Tyr Trp Lys Phe Asn Asn Gln Lys Leu Lys Val Glu Pro Gly
485 490 495

Tyr Pro Lys Ser Ala Leu Arg Asp Trp Met Gly Cys Pro Ser Gly Gly
500 505 510

Arg Pro Asp Glu Gly Thr Glu Glu Glu Thr Glu Val Ile Ile Ile Glu
515 520 525

Val Asp Glu Glu Gly Gly Gly Ala Val Ser Ala Ala Ala Val Val Leu
530 535 540

Pro Val Leu Leu Leu Leu Leu Val Leu Ala Val Gly Leu Ala Val Phe
545 550 555 560

Phe Phe Arg Arg His Gly Thr Pro Arg Arg Leu Leu Tyr Cys Gln Arg
565 570 575

Ser Leu Leu Asp Lys Val
580

<210> 2
<211> 3403
<212> DNA
<213> Homo sapiens

<400> 2
agttcagtgc ctaccgaaga caaaggcgcc ccgagggagt ggcggtgcga ccccagggcg 60
tgggcccggc cgcggagcca cactgcccgg ctgacccggt ggtctcggac catgtctccc 120
gccccaagac cctcccgttg tctcctgctc cccctgctca cgctcggcac cgcgctcgcc 180

tccctcggct cggcccaaag cagcagcttc agccccgaag cctggctaca gcaatatggc 240
tacctgcctc ccggggacct acgtacccac acacagcgct caccccagtc actctcagcg 300
gccatcgctg ccatgcagaa gttttacggc ttgcaagtaa caggcaaagc tgatgcagac 360
accatgaagg ccatgaggcg cccccgatgt ggtgttccag acaagtttgg ggctgagatc 420
aaggccaatg ttcgaaggaa gcgctacgcc atccagggtc tcaaatggca acataatgaa 480
attactttct gcatccagaa ttacacccca aaggtgggcg agtatgccac atacgaggcc 540
attcgcaagg cgttccgcgt gtgggagagt gccacaccac tgcgcttccg cgaggtgccc 600
tatgcctaca tccgtgaggg ccatgagaag caggccgaca tcatgatctt ctttgccgag 660
ggcttccatg gcgacagcac gcccttcgat ggtgagggcg gcttcctggc ccatgcctac 720
ttcccagggc ccaacattgg aggagacacc cactttgact ctgccgagcc ttggactgtc 780
aggaatgagg atctgaatgg aaatgacatc ttcctggtgg ctgtgcacga gctgggccat 840
gccctggggc tcgagcattc cagtgacccc tcggccatca tggcaccctt ttaccagtgg 900
atggacacgg agaattttgt gcttcccgat gatgaccgcc ggggcatcca gcaactttat 960
gggggtgagt cagggttccc caccaagatg ccccctcaac ccaggactac ctcccggcct 1020
tctgttcctg ataaacccaa aaaccccacc tatgggccca acatctgtga cgggaacttt 1080
gacaccgtgg ccatgctccg aggggagatg tttgtcttca agaagcgctg gttctggcgg 1140
gtgaggaata accaagtgat ggatggatac ccaatgccca ttggccagtt ctggcggggc 1200
ctgcctgcgt ccatcaacac tgcctacgag aggaaggatg gcaaattcgt cttcttcaaa 1260
ggagacaagc attgggtgtt tgatgaggcg tccctggaac ctggctaccc caagcacatt 1320
aaggagctgg gccgagggct gcctaccgac aagattgatg ctgctctctt ctggatgccc 1380
aatggaaaga cctacttctt ccgtggaaac aagtactacc gtttcaacga agagctcagg 1440
gcagtggata gcgagtaccc caagaacatc aaagtctggg aagggatccc tgagtctccc 1500
agagggtcat tcatgggcag cgatgaagtc ttcacttact tctacaaggg gaacaaatac 1560
tggaaattca acaaccagaa gctgaaggta gaaccgggct accccaagtc agccctgagg 1620
gactggatgg gctgcccatc gggaggccgg ccggatgagg ggactgagga ggagacggag 1680
gtgatcatca ttgaggtgga cgaggagggc ggcggggcgg tgagcgcggc tgccgtggtg 1740
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cgccatggga cccccaggcg actgctctac tgccagcgtt ccctgctgga caaggtctga 1860
cgcccatccg ccggcccgcc cactcctacc acaaggactt tgcctctgaa ggccagtggc 1920
agcaggtggt ggtgggtggg ctgctcccat cgtcccgagc cccctccccg cagcctcctt 1980
gcttctctct gtcccctggc tggcctcctt caccctgacc gcctccctcc ctcctgcccc 2040
ggcattgcat cttccctaga taggtccccc gagggctgag tgggagggcg gccctttcca 2100
gcctctgccc ctcaggggaa ccctgtagct ttgtgtctgt ccagccccat ctgaatgtgt 2160
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catctgctcc ttttccatcc cctgacatac cttaacctct gaactctgac ctcaggaggc 2280
tctggggaac tccagccctg aaagccccag gtgtacccaa ttggcagcct ctcactactc 2340
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gtggggactg ccaagccacc ctaagacctt gggaggaaaa ctcagagagg gtcttcgttg 2460
ctcagtcagt caagttcctc ggagatcttc ctctgcctca cctaccccag ggaacttcca 2520
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cctctcgaat gttagccttg gatggggctt tcacagttag aagagctgaa accaggggtg 2640
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cttaaggcca cagagaaaga accttgccca aactcaggca gctggggctg aggcccaaag 2760
gcagaacagc cagagggggc aggaggggac caaaaaggaa aatgaggacg tgcagcagca 2820
ttggaaggct ggggcccggc agccaggtta aagctaacag ggggccatca gggtgggctt 2880
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gctgcccagg cagggtggag gggaagggta gggcagccag agaaaggagc agagaaggca 3000
cacaaacgag gaatgagggg cttcacgaga ggccacaggg cctggctggc cacgctgtcc 3060

cggcctgctc accatctcag tgagggacag gagctggggc tgcttaggct gggtccacgc 3120
ttccctggtg ccagcacccc tcaagcctgt ctcaccagtg gcctgccctc tcgctccccc 3180
acccagccca cccattgaag tctccttggg tcccaaaggt gggcatggta ccggggactt 3240
gggagagtga gacccagtgg agggagcaag aggagaggga tgtgggggggg tggggcacgg 3300
gtaggggaaa tggggtgaac ggtgctggca gttcggctag atttctgtct tgtttgtttt 3360
tttgttttgt ttaatgtata tttttattat aattattata tat 3403

<210> 3
<211> 7
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Highly conserved
sequence fragments from MMP family

<400> 3
Pro Arg Cys Gly Val Pro Asp
1 5

<210> 4
<211> 9
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Highly conserved
sequence fragments from MMP family

<400> 4
Gly Asp Ala His Phe Asp Asp Asp Glu
1 5

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 5
ccmmgvtgys gvrwbccwga 20

<210> 6
<211> 25

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 6
ytcrtsvtcr tcraartgrr hrtcy 25

<210> 7
<211> 30
<212> PRT
<213> Homo sapiens

<400> 7
Gly Gly Gly Ala Val Ser Ala Ala Ala Val Val Leu Pro Val Leu Leu
1 5 10 15

Leu Leu Leu Val Leu Ala Val Gly Leu Ala Val Phe Phe Phe
20 25 30

<210> 8
<211> 14
<212> PRT
<213> Homo sapiens

<400> 8
Arg Glu Val Pro Tyr Ala Tyr Ile Arg Glu Gly His Glu Lys
1 5 10

<210> 9
<211> 14
<212> PRT
<213> Homo sapiens

<400> 9
Asp Gly Asn Phe Asp Thr Val Ala Met Leu Arg Gly Glu Met
1 5 10

<210> 10
<211> 15
<212> PRT
<213> Homo sapiens

<400> 10

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Pro Lys Ser Ala Leu Arg Asp Trp Met Gly Cys Pro Ser Gly Gly
 1               5                  10                  15


<210> 11
<211> 489
<212> PRT
<213> Unknown

<220>
<223> X = UNKNOWN

<220>
<223> Description of Unknown Organism: Known Member of
      Matrix Metalloproteinase Family

<400> 11
Met Ala Pro Ala Ala Trp Leu Arg Ser Ala Ala Ala Arg Ala Leu Leu
 1               5                  10                  15

Pro Pro Met Leu Leu Leu Leu Leu Gln Pro Pro Pro Leu Leu Ala Arg
            20                  25                  30

Ala Leu Pro Pro Asp Val His His Leu His Ala Glu Arg Arg Gly Pro
        35                  40                  45

Gln Pro Trp His Ala Ala Leu Pro Ser Ser Pro Ala Pro Ala Pro Ala
    50                  55                  60

Thr Gln Glu Ala Pro Arg Pro Ala Ser Ser Leu Arg Pro Pro Arg Cys
65                  70                  75                  80

Gly Val Pro Asp Pro Ser Asp Gly Leu Ser Ala Arg Asn Arg Gln Lys
                85                  90                  95

Arg Phe Val Leu Ser Gly Gly Arg Trp Glu Lys Thr Asp Leu Thr Tyr
            100                 105                 110

Arg Ile Leu Arg Phe Pro Trp Gln Leu Val Gln Glu Gln Val Arg Gln
        115                 120                 125

Thr Met Ala Glu Ala Leu Lys Val Trp Ser Asp Val Thr Pro Leu Thr
    130                 135                 140

Phe Thr Glu Val His Glu Gly Arg Ala Asp Ile Met Ile Asp Phe Ala
145                 150                 155                 160

Arg Tyr Trp Asp Gly Asp Asp Leu Pro Phe Asp Gly Pro Gly Gly Ile
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                165                 170                 175

Leu Ala His Ala Phe Phe Pro Lys Thr His Arg Glu Gly Asp Val His
            180                 185                 190

Phe Asp Tyr Asp Glu Thr Trp Thr Ile Gly Asp Asp Gln Gly Thr Asp
        195                 200                 205

Leu Leu Gln Val Ala Ala His Glu Phe Gly His Val Leu Gly Leu Gln
    210                 215                 220

His Thr Thr Ala Ala Lys Ala Leu Met Ser Ala Phe Tyr Thr Phe Arg
225                 230                 235                 240

Tyr Pro Leu Ser Leu Ser Pro Asp Asp Cys Arg Gly Val Gln His Leu
                245                 250                 255

Tyr Gly Gln Pro Trp Pro Thr Val Thr Ser Arg Thr Pro Ala Leu Gly
            260                 265                 270

Pro Gln Ala Gly Ile Asp Thr Asn Glu Ile Ala Pro Leu Glu Pro Asp
        275                 280                 285

Ala Pro Pro Asp Ala Cys Glu Ala Ser Phe Asp Ala Val Ser Thr Ile
    290                 295                 300

Arg Gly Glu Leu Phe Phe Phe Lys Ala Gly Phe Val Trp Arg Leu Arg
305                 310                 315                 320

Gly Gly Gln Leu Gln Pro Gly Tyr Pro Ala Leu Ala Ser Arg His Trp
                325                 330                 335

Gln Gly Leu Pro Ser Pro Val Asp Ala Ala Phe Glu Asp Ala Gln Gly
            340                 345                 350

His Ile Trp Phe Phe Gln Gly Ala Gln Tyr Trp Val Tyr Asp Gly Glu
        355                 360                 365

Lys Pro Val Leu Gly Pro Ala Pro Leu Thr Glu Leu Gly Leu Val Arg
    370                 375                 380

Phe Pro Val His Ala Ala Leu Val Trp Gly Pro Glu Lys Asn Lys Ile
385                 390                 395                 400

Tyr Phe Phe Arg Gly Arg Asp Tyr Trp Arg Phe His Pro Ser Thr Arg
                405                 410                 415

Arg Val Asp Ser Pro Val Pro Arg Arg Ala Thr Asp Trp Arg Gly Val
```

420 425 430

Pro Ser Glu Ile Asp Ala Ala Phe Gln Asp Ala Asp Gly Tyr Ala Tyr
435 440 445

Phe Leu Arg Gly Arg Leu Tyr Trp Lys Phe Asp Pro Val Lys Val Lys
450 455 460

Ala Leu Glu Gly Phe Pro Arg Leu Val Gly Pro Asp Phe Phe Gly Cys
465 470 475 480

Ala Glu Pro Ala Asn Thr Phe Leu Xaa
485

<210> 12
<211> 469
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of Matrix Metalloproteinase Family

<400> 12
Met His Ser Phe Pro Pro Leu Leu Leu Leu Leu Phe Trp Gly Val Val
1 5 10 15

Ser His Ser Phe Pro Ala Thr Leu Glu Thr Gln Glu Gln Asp Val Asp
20 25 30

Leu Val Gln Lys Tyr Leu Glu Lys Tyr Tyr Asn Leu Lys Asn Asp Gly
35 40 45

Arg Gln Val Glu Lys Arg Arg Asn Ser Gly Pro Val Val Glu Lys Leu
50 55 60

Lys Gln Met Gln Glu Phe Phe Gly Leu Lys Val Thr Gly Lys Pro Asp
65 70 75 80

Ala Glu Thr Leu Lys Val Met Lys Gln Pro Arg Cys Gly Val Pro Asp
85 90 95

Val Ala Gln Phe Val Leu Thr Glu Gly Asn Pro Arg Trp Glu Gln Thr
100 105 110

His Leu Thr Tyr Arg Ile Glu Asn Tyr Thr Pro Asp Leu Pro Arg Ala
115 120 125

```
Asp Val Asp His Ala Ile Glu Lys Ala Phe Gln Leu Trp Ser Asn Val
    130                 135                 140

Thr Pro Leu Thr Phe Thr Lys Val Ser Glu Gly Gln Ala Asp Ile Met
145                 150                 155                 160

Ile Ser Phe Val Arg Gly Asp His Arg Asp Asn Ser Pro Phe Asp Gly
                165                 170                 175

Pro Gly Gly Asn Leu Ala His Ala Phe Gln Pro Gly Pro Gly Ile Gly
            180                 185                 190

Gly Asp Ala His Phe Asp Glu Asp Glu Arg Trp Thr Asn Asn Phe Thr
        195                 200                 205

Glu Tyr Asn Leu His Arg Val Ala Ala His Glu Leu Gly His Ser Leu
    210                 215                 220

Gly Leu Ser His Ser Thr Asp Ile Gly Ala Leu Met Tyr Pro Ser Tyr
225                 230                 235                 240

Thr Phe Ser Gly Asp Val Gln Leu Ala Gln Asp Asp Ile Asp Gly Ile
                245                 250                 255

Gln Ala Ile Tyr Gly Arg Ser Gln Asn Pro Val Gln Pro Ile Gly Pro
            260                 265                 270

Gln Thr Pro Lys Ala Cys Asp Ser Lys Leu Thr Phe Asp Ala Ile Thr
        275                 280                 285

Thr Ile Arg Gly Glu Val Met Phe Phe Lys Asp Arg Phe Tyr Met Arg
    290                 295                 300

Thr Asn Pro Phe Tyr Pro Glu Val Glu Leu Asn Phe Thr Ser Val Phe
305                 310                 315                 320

Trp Pro Gln Leu Pro Asn Gly Leu Glu Ala Ala Tyr Glu Phe Ala Asp
                325                 330                 335

Arg Asp Glu Val Arg Phe Phe Lys Gly Asn Lys Tyr Trp Ala Val Gln
            340                 345                 350

Gly Gln Asn Val Leu His Gly Tyr Pro Lys Asp Ile Tyr Ser Ser Phe
        355                 360                 365

Gly Phe Pro Arg Thr Val Lys His Ile Asp Ala Ala Leu Ser Glu Glu
    370                 375                 380
```

Asn Thr Gly Lys Thr Tyr Phe Phe Val Ala Asn Lys Tyr Trp Arg Tyr
385 390 395 400

Asp Glu Tyr Lys Arg Ser Met Asp Pro Gly Tyr Pro Lys Met Ile Ala
405 410 415

His Asp Phe Pro Gly Ile Gly His Lys Val Asp Ala Val Phe Met Lys
420 425 430

Asp Gly Phe Phe Tyr Phe Phe His Gly Thr Arg Gln Tyr Lys Phe Asp
435 440 445

Pro Lys Thr Lys Arg Ile Leu Thr Leu Gln Lys Ala Asn Ser Trp Phe
450 455 460

Asn Cys Arg Lys Asn
465

<210> 13
<211> 468
<212> PRT
<213> Unknown

<220>
<223> X = UNKNOWN

<220>
<223> Description of Unknown Organism: Known Member of
Matrix Metalloproteinase Family

<400> 13
Met Phe Ser Leu Lys Thr Leu Pro Phe Leu Leu Leu Leu His Val Gln
1 5 10 15

Ile Ser Lys Ala Phe Pro Val Ser Ser Lys Glu Lys Asn Thr Lys Thr
20 25 30

Val Gln Asp Tyr Leu Glu Lys Phe Tyr Gln Leu Pro Ser Asn Gln Tyr
35 40 45

Gln Ser Thr Arg Lys Asn Gly Thr Asn Val Ile Val Glu Lys Leu Lys
50 55 60

Glu Met Gln Arg Phe Phe Gly Leu Asn Val Thr Gly Lys Pro Asn Glu
65 70 75 80

```
Glu Thr Leu Asp Met Met Lys Lys Pro Arg Cys Gly Val Pro Asp Ser
                85                  90                  95

Gly Gly Phe Met Leu Thr Pro Gly Asn Pro Lys Trp Glu Arg Thr Asn
            100                 105                 110

Leu Thr Tyr Arg Ile Arg Asn Tyr Thr Pro Gln Leu Ser Glu Ala Glu
        115                 120                 125

Val Glu Arg Ala Ile Lys Asp Ala Phe Glu Leu Trp Ser Val Ala Ser
    130                 135                 140

Pro Leu Ile Phe Thr Arg Ile Ser Gln Gly Glu Ala Asp Ile Asn Ile
145                 150                 155                 160

Ala Phe Tyr Gln Arg Asp His Gly Asp Asn Ser Pro Phe Asp Gly Pro
                165                 170                 175

Asn Gly Ile Leu Ala His Ala Phe Gln Pro Gly Gln Gly Ile Gly Gly
            180                 185                 190

Asp Ala His Phe Asp Ala Glu Glu Thr Trp Thr Asn Thr Ser Ala Asn
        195                 200                 205

Tyr Asn Leu Phe Leu Val Ala Ala His Glu Phe Gly His Ser Leu Gly
    210                 215                 220

Leu Ala His Ser Ser Asp Pro Gly Ala Leu Met Tyr Pro Asn Tyr Ala
225                 230                 235                 240

Phe Arg Glu Thr Ser Asn Tyr Ser Leu Pro Gln Asp Asp Ile Asp Gly
                245                 250                 255

Ile Gln Ala Ile Tyr Gly Leu Ser Ser Asn Pro Ile Gln Pro Thr Gly
            260                 265                 270

Pro Ser Thr Pro Lys Pro Cys Asp Pro Ser Leu Thr Phe Asp Ala Ile
        275                 280                 285

Thr Thr Leu Arg Gly Glu Ile Leu Phe Phe Lys Asp Arg Tyr Phe Trp
    290                 295                 300

Arg Arg His Pro Gln Leu Gln Arg Val Glu Met Asn Phe Ile Ser Leu
305                 310                 315                 320

Phe Trp Pro Ser Leu Pro Thr Gly Ile Gln Ala Ala Tyr Glu Asp Phe
                325                 330                 335
```

Asp Arg Asp Leu Ile Phe Leu Phe Lys Gly Asn Gln Tyr Trp Ala Leu
340 345 350

Ser Gly Tyr Asp Ile Leu Gln Gly Tyr Pro Lys Asp Ile Ser Asn Tyr
355 360 365

Gly Phe Pro Ser Ser Val Gln Ala Ile Asp Ala Ala Val Phe Tyr Arg
370 375 380

Ser Lys Thr Tyr Phe Phe Val Asn Asp Gln Phe Trp Arg Tyr Asp Asn
385 390 395 400

Gln Arg Gln Phe Met Glu Pro Gly Tyr Pro Lys Ser Ile Ser Gly Ala
405 410 415

Phe Pro Gly Ile Glu Ser Lys Val Asp Ala Val Phe Gln Gln Glu His
420 425 430

Phe Phe His Val Phe Ser Gly Pro Arg Tyr Tyr Ala Phe Asp Leu Ile
435 440 445

Ala Gln Arg Val Thr Arg Val Ala Arg Gly Asn Lys Trp Leu Asn Cys
450 455 460

Arg Tyr Gly Xaa
465

<210> 14
<211> 476
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of Matrix Metalloproteinase Family

<400> 14
Met Met His Leu Ala Phe Leu Val Leu Leu Cys Leu Pro Val Cys Ser
1 5 10 15

Ala Tyr Pro Leu Ser Gly Ala Ala Lys Glu Glu Asp Ser Asn Lys Asp
20 25 30

Leu Ala Gln Gln Tyr Leu Glu Lys Tyr Tyr Asn Leu Glu Lys Asp Val
35 40 45

Lys Gln Phe Arg Arg Lys Asp Ser Asn Leu Ile Val Lys Lys Ile Gln

50 55 60

Gly Met Gln Lys Phe Leu Gly Leu Glu Val Thr Gly Lys Leu Asp Thr
65 70 75 80

Asp Thr Leu Glu Val Met Arg Lys Pro Arg Cys Gly Val Pro Asp Val
85 90 95

Gly His Phe Ser Ser Phe Pro Gly Met Pro Lys Trp Arg Lys Thr His
100 105 110

Leu Thr Tyr Arg Ile Val Asn Tyr Thr Pro Asp Leu Pro Arg Asp Ala
115 120 125

Val Asp Ser Ala Ile Glu Lys Ala Leu Lys Val Trp Glu Glu Val Thr
130 135 140

Pro Leu Thr Phe Ser Arg Leu Tyr Glu Gly Glu Ala Asp Ile Met Ile
145 150 155 160

Ser Phe Ala Val Lys Glu His Gly Asp Phe Tyr Ser Phe Asp Gly Pro
165 170 175

Gly His Ser Leu Ala His Ala Tyr Pro Pro Gly Pro Gly Leu Tyr Gly
180 185 190

Asp Ile His Phe Asp Asp Asp Glu Lys Trp Thr Glu Asp Ala Ser Gly
195 200 205

Thr Asn Leu Phe Leu Val Ala Ala His Glu Leu Gly His Ser Leu Gly
210 215 220

Leu Phe His Ser Ala Asn Thr Glu Ala Leu Met Tyr Pro Leu Tyr Asn
225 230 235 240

Ser Phe Thr Glu Leu Ala Gln Phe Arg Leu Ser Gln Asp Asp Val Asn
245 250 255

Gly Ile Gln Ser Leu Tyr Gly Pro Pro Pro Ala Ser Thr Glu Glu Pro
260 265 270

Leu Val Pro Thr Lys Ser Val Pro Ser Gly Ser Glu Met Pro Ala Lys
275 280 285

Cys Asp Pro Ala Leu Ser Phe Asp Ala Ile Ser Thr Leu Arg Gly Glu
290 295 300

Tyr Leu Phe Phe Lys Asp Arg Tyr Phe Trp Arg Arg Ser His Trp Asn

305 310 315 320

Pro Glu Pro Glu Phe His Leu Ile Ser Ala Phe Trp Pro Ser Leu Pro
325 330 335

Ser Tyr Leu Asp Ala Ala Tyr Glu Val Asn Ser Arg Asp Thr Val Phe
340 345 350

Ile Phe Lys Gly Asn Glu Phe Trp Ala Ile Arg Gly Asn Glu Val Gln
355 360 365

Ala Gly Tyr Pro Arg Gly Ile His Thr Leu Gly Phe Pro Pro Thr Ile
370 375 380

Arg Lys Ile Asp Ala Ala Val Ser Asp Lys Glu Lys Lys Lys Thr Tyr
385 390 395 400

Phe Phe Ala Ala Asp Lys Tyr Trp Arg Phe Asp Glu Asn Ser Gln Ser
405 410 415

Met Glu Gln Gly Phe Pro Arg Leu Ile Ala Asp Asp Phe Pro Gly Val
420 425 430

Glu Pro Lys Val Asp Ala Val Leu Gln Ala Phe Gly Phe Phe Tyr Phe
435 440 445

Phe Ser Gly Ser Ser Gln Phe Glu Phe Asp Pro Asn Ala Arg Met Val
450 455 460

Thr His Ile Leu Lys Ser Asn Ser Trp Leu His Cys
465 470 475

<210> 15
<211> 477
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of
Matrix Metalloproteinase Family

<400> 15
Met Lys Ser Leu Pro Ile Leu Leu Leu Leu Cys Val Ala Val Cys Ser
1 5 10 15

Ala Tyr Pro Leu Asp Gly Ala Ala Arg Gly Glu Asp Thr Ser Met Asn
20 25 30

Leu Val Gln Lys Tyr Leu Glu Asn Tyr Tyr Asp Leu Lys Lys Asp Val
35 40 45

Lys Gln Phe Val Arg Arg Lys Asp Ser Gly Pro Val Val Lys Lys Ile
50 55 60

Arg Glu Met Gln Lys Phe Leu Gly Leu Glu Val Thr Gly Lys Leu Asp
65 70 75 80

Ser Asp Thr Leu Glu Val Met Arg Lys Pro Arg Cys Gly Val Pro Asp
85 90 95

Val Gly His Phe Arg Thr Phe Pro Gly Ile Pro Lys Trp Arg Lys Thr
100 105 110

His Leu Thr Tyr Arg Ile Val Asn Tyr Thr Pro Asp Leu Pro Lys Asp
115 120 125

Ala Val Asp Ser Ala Val Glu Lys Ala Leu Lys Val Trp Glu Glu Val
130 135 140

Thr Pro Leu Thr Phe Ser Arg Leu Tyr Glu Gly Glu Ala Asp Ile Met
145 150 155 160

Ile Ser Phe Ala Val Arg Glu His Gly Asp Phe Tyr Pro Phe Asp Gly
165 170 175

Pro Gly Asn Val Leu Ala His Ala Tyr Ala Pro Gly Pro Gly Ile Asn
180 185 190

Gly Asp Ala His Phe Asp Asp Asp Glu Gln Trp Thr Lys Asp Thr Thr
195 200 205

Gly Thr Asn Leu Phe Leu Val Ala Ala His Glu Ile Gly His Ser Leu
210 215 220

Gly Leu Phe His Ser Ala Asn Thr Glu Ala Leu Met Tyr Pro Leu Tyr
225 230 235 240

His Ser Leu Thr Asp Leu Thr Arg Phe Arg Leu Ser Gln Asp Asp Ile
245 250 255

Asn Gly Ile Gln Ser Leu Tyr Gly Pro Pro Pro Asp Ser Pro Glu Thr
260 265 270

Pro Leu Val Pro Thr Glu Pro Val Pro Pro Glu Pro Gly Thr Pro Ala
275 280 285

```
Asn Cys Asp Pro Ala Leu Ser Phe Asp Ala Val Ser Thr Leu Arg Gly
    290                 295                 300

Glu Ile Leu Ile Phe Lys Asp Arg His Phe Trp Arg Lys Ser Leu Arg
305                 310                 315                 320

Lys Leu Glu Pro Glu Leu His Leu Ile Ser Ser Phe Trp Pro Ser Leu
                325                 330                 335

Pro Ser Gly Val Asp Ala Ala Tyr Glu Val Thr Ser Lys Asp Leu Val
            340                 345                 350

Phe Ile Phe Lys Gly Asn Gln Phe Trp Ala Ile Arg Gly Asn Glu Val
        355                 360                 365

Arg Ala Gly Tyr Pro Arg Gly Ile His Thr Leu Gly Phe Pro Pro Thr
    370                 375                 380

Val Arg Lys Ile Asp Ala Ala Ile Ser Asp Lys Glu Lys Asn Lys Thr
385                 390                 395                 400

Tyr Phe Phe Val Glu Asp Lys Tyr Trp Arg Phe Asp Glu Lys Arg Asn
                405                 410                 415

Ser Met Glu Pro Gly Phe Pro Lys Gln Ile Ala Glu Asp Phe Pro Gly
            420                 425                 430

Ile Asp Ser Lys Ile Asp Ala Val Phe Glu Glu Phe Gly Phe Phe Tyr
        435                 440                 445

Phe Phe Thr Gly Ser Ser Gln Leu Glu Phe Asp Pro Asn Ala Lys Lys
    450                 455                 460

Val Thr His Thr Leu Lys Ser Asn Ser Trp Leu Asn Cys
465                 470                 475
```

<210> 16
<211> 708
<212> PRT
<213> Unknown

<220>
<223> X = UNKNOWN

<220>
<223> Description of Unknown Organism: Known Member of

Matrix Metalloproteinase Family

<400> 16

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Met Ser Leu Trp Gln Pro Leu Val Leu Val Leu Leu Val Leu Gly Cys
 1               5                  10                  15

Cys Phe Ala Ala Pro Arg Gln Arg Gln Ser Thr Leu Val Leu Phe Pro
            20                  25                  30

Gly Asp Leu Arg Thr Asn Leu Thr Asp Arg Gln Leu Ala Glu Glu Tyr
        35                  40                  45

Leu Tyr Arg Tyr Gly Tyr Thr Arg Val Ala Glu Met Arg Gly Glu Ser
    50                  55                  60

Lys Ser Leu Gly Pro Ala Leu Leu Leu Leu Gln Lys Gln Leu Ser Leu
65                  70                  75                  80

Pro Glu Thr Gly Glu Leu Asp Ser Ala Thr Leu Lys Ala Met Arg Thr
                85                  90                  95

Pro Arg Cys Gly Val Pro Asp Leu Gly Arg Phe Gln Thr Phe Glu Gly
            100                 105                 110

Asp Leu Lys Trp His His His Asn Ile Thr Tyr Trp Ile Gln Asn Tyr
        115                 120                 125

Ser Glu Asp Leu Pro Arg Ala Val Ile Asp Asp Ala Phe Ala Arg Ala
    130                 135                 140

Phe Ala Leu Trp Ser Ala Val Thr Pro Leu Thr Phe Thr Arg Val Tyr
145                 150                 155                 160

Ser Arg Asp Ala Asp Ile Val Ile Gln Phe Gly Val Ala Glu His Gly
                165                 170                 175

Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly Leu Leu Ala His Ala Phe
            180                 185                 190

Pro Pro Gly Pro Gly Ile Gln Gly Asp Ala His Phe Asp Asp Asp Glu
        195                 200                 205

Leu Trp Ser Leu Gly Lys Gly Val Val Val Pro Thr Arg Phe Gly Asn
    210                 215                 220

Ala Asp Gly Ala Ala Cys His Phe Pro Phe Ile Phe Glu Gly Arg Ser
225                 230                 235                 240
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Tyr Ser Ala Cys Thr Thr Asp Gly Arg Ser Asp Gly Leu Pro Trp Cys
                245                 250                 255

Ser Thr Thr Ala Asn Tyr Asp Thr Asp Asp Arg Phe Gly Phe Cys Pro
            260                 265                 270

Ser Glu Arg Leu Tyr Thr Arg Asp Gly Asn Ala Asp Gly Lys Pro Cys
        275                 280                 285

Gln Phe Pro Phe Ile Phe Gln Gly Gln Ser Tyr Ser Ala Cys Thr Thr
    290                 295                 300

Asp Gly Arg Ser Asp Gly Tyr Arg Trp Cys Ala Thr Thr Ala Asn Tyr
305                 310                 315                 320

Asp Arg Asp Lys Leu Phe Gly Phe Cys Pro Thr Arg Ala Asp Ser Thr
                325                 330                 335

Val Met Gly Gly Asn Ser Ala Gly Glu Leu Cys Val Phe Pro Phe Thr
            340                 345                 350

Phe Leu Gly Lys Glu Tyr Ser Thr Cys Thr Ser Glu Gly Arg Gly Asp
        355                 360                 365

Gly Arg Leu Trp Cys Ala Thr Thr Ser Asn Phe Asp Ser Asp Lys Lys
    370                 375                 380

Trp Gly Phe Cys Pro Asp Gln Gly Tyr Ser Leu Phe Leu Val Ala Ala
385                 390                 395                 400

His Glu Phe Gly His Ala Leu Gly Leu Asp His Ser Ser Val Pro Glu
                405                 410                 415

Ala Leu Met Tyr Pro Met Tyr Arg Phe Thr Glu Gly Pro Pro Leu His
            420                 425                 430

Lys Asp Asp Val Asn Gly Ile Arg His Leu Tyr Gly Pro Arg Pro Glu
        435                 440                 445

Pro Glu Pro Arg Pro Pro Thr Thr Thr Thr Pro Gln Pro Thr Ala Pro
    450                 455                 460

Pro Thr Val Cys Pro Thr Gly Pro Pro Thr Val His Pro Ser Glu Arg
465                 470                 475                 480

Pro Thr Ala Gly Pro Thr Gly Pro Pro Ser Ala Gly Pro Thr Gly Pro
                485                 490                 495
```

Pro Thr Ala Gly Pro Ser Thr Ala Thr Thr Val Pro Leu Ser Pro Val
500 505 510

Asp Asp Ala Cys Asn Val Asn Ile Phe Asp Ala Ile Ala Glu Ile Gly
515 520 525

Asn Gln Leu Tyr Leu Phe Lys Asp Gly Lys Tyr Trp Arg Phe Ser Glu
530 535 540

Gly Arg Gly Ser Arg Pro Gln Gly Pro Phe Leu Ile Ala Asp Lys Trp
545 550 555 560

Pro Ala Leu Pro Arg Lys Leu Asp Ser Val Phe Glu Glu Pro Leu Ser
565 570 575

Lys Lys Leu Phe Phe Phe Ser Gly Arg Gln Val Trp Val Tyr Thr Gly
580 585 590

Ala Ser Val Leu Gly Pro Arg Arg Leu Asp Lys Leu Gly Leu Gly Ala
595 600 605

Asp Val Ala Gln Val Thr Gly Ala Leu Arg Ser Gly Arg Gly Lys Met
610 615 620

Leu Leu Phe Ser Gly Arg Arg Leu Trp Arg Phe Asp Val Lys Ala Gln
625 630 635 640

Met Val Asp Pro Arg Ser Ala Ser Glu Val Asp Arg Met Phe Pro Gly
645 650 655

Val Pro Leu Asp Thr His Asp Val Phe Gln Tyr Arg Glu Lys Ala Tyr
660 665 670

Phe Cys Gln Asp Arg Phe Tyr Trp Arg Val Ser Ser Arg Ser Glu Leu
675 680 685

Asn Gln Val Asp Gln Val Gly Tyr Val Thr Tyr Asp Ile Leu Gln Cys
690 695 700

Pro Glu Asp Xaa
705

<210> 17
<211> 631
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of
Matrix Metalloproteinase Family

<400> 17

```
Ala Pro Ser Pro Ile Ile Lys Phe Pro Gly Asp Val Ala Pro Lys Thr
 1               5                  10                  15

Asp Lys Glu Leu Ala Val Gln Tyr Leu Asn Thr Phe Tyr Gly Cys Pro
            20                  25                  30

Lys Glu Ser Cys Asn Leu Phe Val Leu Lys Asp Thr Leu Lys Lys Met
        35                  40                  45

Gln Lys Phe Phe Gly Leu Pro Gln Thr Gly Asp Leu Asp Gln Asn Thr
    50                  55                  60

Ile Glu Thr Met Arg Lys Pro Arg Cys Gly Asn Pro Asp Val Ala Asn
65                  70                  75                  80

Tyr Asn Phe Phe Pro Arg Lys Pro Lys Trp Asp Lys Asn Gln Ile Thr
                85                  90                  95

Tyr Arg Ile Ile Gly Tyr Thr Pro Asp Leu Asp Pro Glu Thr Val Asp
            100                 105                 110

Asp Ala Phe Ala Arg Ala Phe Gln Val Trp Ser Asp Val Thr Pro Leu
        115                 120                 125

Arg Phe Ser Arg Ile His Asp Gly Glu Ala Asp Ile Met Ile Asn Phe
    130                 135                 140

Gly Arg Trp Glu His Gly Asp Gly Tyr Pro Phe Asp Gly Lys Asp Gly
145                 150                 155                 160

Leu Leu Ala His Ala Phe Ala Pro Gly Thr Gly Val Gly Gly Asp Ser
                165                 170                 175

His Phe Asp Asp Asp Glu Leu Trp Thr Leu Gly Glu Gly Gln Val Val
            180                 185                 190

Arg Val Lys Tyr Gly Asn Ala Asp Gly Glu Tyr Cys Lys Phe Pro Phe
        195                 200                 205

Leu Phe Asn Gly Lys Glu Tyr Asn Ser Cys Thr Asp Thr Gly Arg Ser
    210                 215                 220

Asp Gly Phe Leu Trp Cys Ser Thr Thr Tyr Asn Phe Glu Lys Asp Gly
```

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225                 230                 235                 240

Lys Tyr Gly Phe Cys Pro His Glu Ala Leu Phe Thr Met Gly Gly Asn
                245                 250                 255

Ala Glu Gly Gln Pro Cys Lys Phe Pro Phe Arg Phe Gln Gly Thr Ser
            260                 265                 270

Tyr Asp Ser Cys Thr Thr Glu Gly Arg Thr Asp Gly Tyr Arg Trp Cys
        275                 280                 285

Gly Thr Thr Glu Asp Tyr Asp Arg Asp Lys Lys Tyr Gly Phe Cys Pro
    290                 295                 300

Glu Thr Ala Met Ser Thr Val Gly Gly Asn Ser Glu Gly Ala Pro Cys
305                 310                 315                 320

Val Phe Pro Phe Thr Phe Leu Gly Asn Lys Tyr Glu Ser Cys Thr Ser
                325                 330                 335

Ala Gly Arg Ser Asp Gly Lys Met Trp Cys Ala Thr Thr Ala Asn Tyr
            340                 345                 350

Asp Asp Asp Arg Lys Trp Gly Phe Cys Pro Asp Gln Gly Tyr Ser Leu
        355                 360                 365

Phe Leu Val Ala Ala His Glu Phe Gly His Ala Met Gly Leu Glu His
    370                 375                 380

Ser Gln Asp Pro Gly Ala Leu Met Ala Pro Ile Tyr Thr Tyr Thr Lys
385                 390                 395                 400

Asn Phe Arg Leu Ser Gln Asp Asp Ile Lys Gly Ile Gln Glu Leu Tyr
                405                 410                 415

Gly Ala Ser Pro Asp Ile Asp Leu Gly Thr Gly Pro Thr Pro Thr Leu
            420                 425                 430

Gly Pro Val Thr Pro Glu Ile Cys Lys Gln Asp Ile Val Phe Asp Gly
        435                 440                 445

Ile Ala Gln Ile Arg Gly Glu Ile Phe Phe Phe Lys Asp Arg Phe Ile
    450                 455                 460

Trp Arg Thr Val Thr Pro Arg Asp Lys Pro Met Gly Pro Leu Leu Val
465                 470                 475                 480

Ala Thr Phe Trp Pro Glu Leu Pro Glu Lys Ile Asp Ala Val Tyr Glu
```

485 490 495

Ala Pro Gln Glu Glu Lys Ala Val Phe Phe Ala Gly Asn Glu Tyr Trp
500 505 510

Ile Tyr Ser Ala Ser Thr Leu Glu Arg Gly Tyr Pro Lys Pro Leu Thr
515 520 525

Ser Leu Gly Leu Pro Pro Asp Val Gln Arg Val Asp Ala Ala Phe Asn
530 535 540

Trp Ser Lys Asn Lys Lys Thr Tyr Ile Phe Ala Gly Asp Lys Phe Trp
545 550 555 560

Arg Tyr Asn Glu Val Lys Lys Lys Met Asp Pro Gly Phe Pro Lys Leu
565 570 575

Ile Ala Asp Ala Trp Asn Ala Ile Pro Asp Asn Leu Asp Ala Val Val
580 585 590

Asp Leu Gln Gly Gly Gly His Ser Tyr Phe Phe Lys Gly Ala Tyr Tyr
595 600 605

Leu Lys Leu Glu Asn Gln Ser Leu Lys Ser Val Lys Phe Gly Ser Ile
610 615 620

Lys Ser Asp Trp Leu Gly Cys
625 630

<210> 18
<211> 267
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of
Matrix Metalloproteinase Family

<400> 18
Met Arg Leu Thr Val Leu Cys Ala Val Cys Leu Leu Pro Gly Ser Leu
1 5 10 15

Ala Leu Pro Leu Pro Gln Glu Ala Gly Gly Met Ser Glu Leu Gln Trp
20 25 30

Glu Gln Ala Gln Asp Tyr Leu Lys Arg Phe Tyr Leu Tyr Asp Ser Glu
35 40 45

```
Thr Lys Asn Ala Asn Ser Leu Glu Ala Lys Leu Lys Glu Met Gln Lys
 50                  55                  60

Phe Phe Gly Leu Pro Ile Thr Gly Met Leu Asn Ser Arg Val Ile Glu
65                  70                  75                  80

Ile Met Gln Lys Pro Arg Cys Gly Val Pro Asp Val Ala Glu Tyr Ser
                85                  90                  95

Leu Phe Pro Asn Ser Pro Lys Trp Thr Ser Lys Val Val Thr Tyr Arg
            100                 105                 110

Ile Val Ser Tyr Thr Arg Asp Leu Pro His Ile Thr Val Asp Arg Leu
        115                 120                 125

Val Ser Lys Ala Leu Asn Met Trp Gly Lys Glu Ile Pro Leu His Phe
    130                 135                 140

Arg Lys Val Val Trp Gly Thr Ala Asp Ile Met Ile Gly Phe Ala Arg
145                 150                 155                 160

Gly Ala His Gly Asp Ser Tyr Pro Phe Asp Gly Pro Gly Asn Thr Leu
                165                 170                 175

Ala His Ala Phe Ala Pro Gly Thr Gly Leu Gly Gly Asp Ala His Phe
            180                 185                 190

Asp Glu Asp Glu Arg Trp Thr Asp Gly Ser Ser Leu Gly Ile Asn Phe
        195                 200                 205

Leu Tyr Ala Ala Thr His Glu Leu Gly His Ser Leu Gly Met Gly His
    210                 215                 220

Ser Ser Asp Pro Asn Ala Val Met Tyr Pro Thr Tyr Gly Asn Gly Asp
225                 230                 235                 240

Pro Gln Asn Phe Lys Leu Ser Gln Asp Asp Ile Lys Gly Ile Gln Lys
                245                 250                 255

Leu Tyr Gly Lys Arg Ser Asn Ser Arg Lys Lys
            260                 265
```

<210> 19
<211> 231
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism: Known Member of
Matrix Metalloproteinase Family

<400> 19

```
Met Pro Leu Leu Leu Leu Leu Glu Tyr Leu Glu Lys Leu Met Gln Lys
 1               5                  10                  15

Phe Gly Leu Val Thr Gly Lys Leu Asp Thr Leu Met Arg Lys Pro Arg
            20                  25                  30

Cys Gly Val Pro Asp Val Gly Phe Phe Pro Gly Pro Lys Trp Thr Leu
        35                  40                  45

Thr Tyr Arg Ile Asn Tyr Thr Pro Asp Leu Pro Val Asp Ala Lys Ala
    50                  55                  60

Phe Val Trp Ser Val Thr Pro Leu Thr Phe Arg Val Glu Gly Ala Asp
65                  70                  75                  80

Ile Met Ile Phe Ala His Gly Asp Tyr Pro Phe Asp Gly Pro Gly Gly
                85                  90                  95

Leu Ala His Ala Phe Pro Gly Pro Gly Ile Gly Gly Asp Ala His Phe
            100                 105                 110

Asp Asp Glu Trp Thr Asn Leu Phe Leu Val Ala Ala His Glu Gly His
        115                 120                 125

Ser Leu Gly Leu His Ser Asp Pro Ala Leu Met Tyr Pro Thr Phe Phe
    130                 135                 140

Leu Ser Gln Asp Asp Ile Gly Ile Gln Leu Tyr Gly Pro Pro Thr Cys
145                 150                 155                 160

Asp Phe Asp Ala Ile Thr Arg Gly Glu Phe Phe Lys Asp Arg Trp Arg
                165                 170                 175

Leu Ser Phe Trp Pro Leu Pro Asp Ala Ala Tyr Glu Phe Phe Gly Asn
            180                 185                 190

Tyr Trp Gly Gly Tyr Pro Ile Leu Gly Pro Val Asp Ala Ala Lys Thr
        195                 200                 205

Tyr Phe Phe Lys Trp Arg Asp Met Pro Gly Pro Ile Phe Pro Gly Asp
    210                 215                 220
```

Ala Val Phe Phe Trp Leu Cys
225 230